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**Importance of diapause aggregations for the
reproduction of ladybird *hippodamia undecimnotata*
(Schneider) : (Coleoptera Coccinellidae)**

Eline Susset

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Université Fédérale



Toulouse Midi-Pyrénées

THÈSE

En vue de l'obtention du

DOCTORAT DE L'UNIVERSITÉ DE TOULOUSE

Délivré par :

Université Toulouse 3 Paul Sabatier (UT3 Paul Sabatier)

Présentée et soutenue par :

Eline Susset

le jeudi 3 novembre 2016

Titre :

Importance des agrégations de diapause dans la reproduction de la coccinelle
Hippodamia undecimnotata (Schneider) (Coleoptera: Coccinellidae)

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“The Earth does not belong to man; man belongs to the Earth. This we know. All things are connected like the blood which unites one family. Whatever befalls the Earth befalls the sons of the Earth. Man did not weave the web of life, he is merely a strand in it. Whatever he does to the web, he does to himself.”

Chief Seattle (1854)

Avant-propos

Cette thèse consacrée à l'étude des mécanismes ultimes de formation des agrégations de diapause chez les coccinelles a été réalisée au sein du laboratoire Evolution et Diversité Biologique de Toulouse (UMR5174 UPS/CNRS/ENFA).

Elle est constituée d'une introduction générale, de 4 chapitres présentés sous la forme d'articles scientifiques et d'une discussion générale. La bibliographie générale commune à l'introduction générale et à la conclusion générale est placée à la fin du manuscrit.

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*A tous ceux partis trop tôt...
A tous ceux partis pendant cette thèse, et à qui je n'ai pas pu dire au revoir.*

Résumé de thèse

Au cours de leur vie, de nombreux animaux expérimentent la vie en groupe. Si les causes évolutives de certains groupes sont bien identifiées, ce n'est pas le cas de tous, et notamment des agrégations de diapause des arthropodes terrestres (rassemblements monospécifiques d'adultes dans des sites à localisation constante pendant plusieurs mois consécutifs). Dans cette thèse, j'ai testé si, selon l'hypothèse du lek caché, les arthropodes se rassemblent pour trouver leurs partenaires sexuels chez la coccinelle *Hippodamia undecimnotata*. A l'aide d'un modèle de distribution d'espèces, j'ai montré que les coccinelles se rassemblent dans des endroits avec un repère proéminent et où le risque de pesticides est faible. Puis j'ai mis en évidence que les conditions abiotiques dans les sites d'agrégations sont défavorables à la survie des coccinelles. Dans un troisième temps, j'ai mis en évidence l'occurrence d'accouplements dans les sites d'agrégation. Les événements de copulation sont très nombreux. Enfin, j'ai montré que ces accouplements sont peu coûteux en énergie pour les femelles et permettent aux coccinelles de conserver de l'énergie pour les comportements post- agrégation. Cette thèse atteste que les agrégations de diapause font partie du système de reproduction des arthropodes et que la sélection sexuelle peut être l'un des moteurs de la formation des agrégations de diapause.

Mots clés : Coccinellidae, agrégation, diapause, reproduction, réserves énergétiques, conditions abiotiques, SDM

Abstract

Many animals live in groups during part or their whole life. While the evolutionary significance of some kinds of group living are well-known, some others remain poorly known although widespread. A good example is given by the diapause aggregations in terrestrial arthropods, which are monospecific clusters formed at the same places year after. In this thesis, I tested if the arthropods form diapause aggregations to find their mates according to the hidden lek hypothesis with the ladybird *Hippodamia undecimnotata*. By using a Species Distribution Model, I showed that ladybirds aggregate in places with a prominent object and where the risk of being sprayed by pesticides is low. Then, I highlight that abiotic conditions at the nearest of the aggregations are unfavourable to ladybirds' survival. In a third part, I found that mating is widespread activity in the aggregation sites. Finally, I showed that energetic costs linked to mating are low, and thus allow the ladybirds to disperse and display post aggregations behaviour. This thesis shows that the diapause aggregations are part of the mating system of the arthropods and that sexual selection can be a driver of the evolution of diapause aggregations.

Key-words: Coccinellidae, aggregation, diapause, reproduction, energetic reserves, abiotic conditions, SDM

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Introduction générale

La vie en groupe chez les arthropodes : des espèces solitaires à l'eusocialité

Une grande majorité d'animaux expérimente le passage par la vie en groupe au cours de leur vie (Danchin et al. 2008). Ce passage peut être temporaire ou durable, inorganisé ou fortement structuré, liées à des activités aussi diverses que l'approvisionnement, le repos, la reproduction, intervenir à des stades mûres ou immatures et reposer sur un degré plus ou moins important d'interactions (Parrish & Edelman-Keshet 1999 ; Krause & Ruxton 2002). Meute, bande, nuée, troupeau, essaim, banc, horde, harde, colonie et bien d'autres... sont autant de termes employés pour nommer les différents types de groupe et sont le témoignage de la diversité des situations (Krause & Ruxton 2002). Afin de différencier les différents niveaux d'interaction et de classer toutes ces formes de vie en groupe, Crespi & Yanega (1995) et Crespi & Choe (1997) ont proposé une gradation en 5 catégories : espèces solitaires, espèces coloniales, espèces communautaires, espèces à reproduction coopérative, espèces eusociales.

Chez les arthropodes, représentants de la composante majeure de la diversité en termes de richesse spécifique, avec 1 à 5 millions d'espèces (e.g. Mora et al. 2011), toutes les différentes de vie en groupe sont représentées (Crespi & Choe 1997). Parmi elles, la forme de socialité la mieux connue est l'eusocialité, avec des représentants tels que les fourmis, les termites, les abeilles (Hymenoptera) (Michener 1969 ; Wilson 1971). L'eusocialité, transition évolutive majeure (Maynard Smith & Szathmáry 1995), a été source d'intérêt pour de nombreux scientifiques. C'est la forme la plus avancée de la vie en groupe. Elle implique la division du travail, la coopération, le chevauchement de générations, les prises de décisions collective et les soins aux jeunes (Michener 1969 ; Wilson 1971 ; Crespi & Yanega 1995). De plus, elle implique que les individus apparentés sont répartis en castes : une caste d'individus reproducteurs et une caste d'individus stériles, qui donnent des soins aux descendants de la première caste.

Darwin (1859) fut le premier à être déconcerté par l'évolution d'un tel comportement reproductif altruiste : pourquoi des individus renonceraient-ils à la reproduction au profit d'autres individus ? Bien que ne disposant pas encore de connaissances génétiques approfondies, il pensa tout de même à une forme de sélection au niveau de la famille ou du

groupe (Darwin 1859). Ce n'est qu'un siècle plus tard, grâce aux travaux d'Haldane (1955) puis à ceux d'Hamilton (1964), que la communauté scientifique découvrit l'importance de l'apparentement dans l'évolution et le maintien de ce comportement. Hamilton (1964) proposa l'hypothèse de la sélection de parentèle selon laquelle la coopération est favorisée par la sélection naturelle lorsque le ratio coûts/bénéfices du comportement altruiste est inférieur au degré d'apparentement des individus. Cette condition est représentée par l'équation :

$$r.B > C$$

où r représente le coefficient d'apparentement, B le bénéfice pour le receveur, C le coût pour le donneur.

Bien que récemment critiqués par Wilson et al. (2005) et par Nowak et al. (2010), les concepts de sélection de parentèle et de valeur sélective inclusive¹ continuent d'avoir de nombreux partisans et restent les références concernant l'évolution de la coopération et de l'eusocialité (Abbot et al. 2011 ; Liao et al. 2015). Ainsi, les travaux d'Hamilton (1964) offrent le cadre théorique concernant les mécanismes évolutifs de l'apparition et du maintien de l'eusocialité.

De l'autre côté de l'échelle de socialité établie par Crespi & Yanega (1995) et Crespi & Choe (1997), on retrouve un grand nombre d'espèces qui forment des agrégations (définition dans Encadré 1) temporaires, notamment en réponse aux conditions environnementales saisonnières. Représentant un degré peu élaboré de socialité, ces rassemblements restent mal connus d'un point de vue évolutif. Or la compréhension de l'évolution des formes de vie en groupe peu élaborées est d'une importance cruciale car elle peut amener à comprendre les premières étapes de la vie en groupe, les pressions de sélection qui entraînent les animaux à former des groupes temporaires, puis des groupes cohérents et persistants et ainsi identifier les étapes qui ont mené à l'évolution de l'eusocialité (Danforth 2002; Agnarsson 2006 ; Lubin & Bilde 2007 ; Pinto da rocha et al. 2007).

¹ La valeur sélective inclusive fait référence à la valeur sélective d'un individu en prenant en compte les conséquences de ses actions sur sa propre reproduction et celle des individus auxquels il est génétiquement lié.

Encadré 1 : Définition de l'agrégation

Pour comprendre l'évolution de la vie en groupe, il convient de définir ce qu'est l'agrégation. Dans cette thèse, je me base sur le raisonnement formulé par Danchin et al. (2008) et définissons l'agrégation par rapport à la distribution idéale libre.

La **distribution idéale libre** est une théorie développée par Stephen Fretwell et Henry Lucas (1970) pour expliquer la distribution spatiale des animaux parmi leurs habitats. Elle postule que les ressources sont distribuées de manière hétérogène et sont constantes dans le temps (Fretwell & Lucas, 1970). C'est un modèle théorique définissant la distribution à l'équilibre d'une population d'individus dans un habitat hétérogène contenant plusieurs parcelles de qualités différentes. Le modèle de base considère que tous les individus ont la même capacité compétitive, connaissent parfaitement la qualité des parcelles de l'environnement (d'où le qualificatif idéal) et sont capables de se déplacer sans aucun coût entre les parcelles de cet environnement (d'où le qualificatif de libre). A l'équilibre, les individus ont tous la même aptitude moyenne quelle que soit la parcelle qu'ils exploitent.

L'agrégation est la distribution biaisée des individus dans l'espace et dans le temps par rapport au cadre théorique de la distribution libre idéale. Elle diffère de la distribution idéale libre dans le sens où la distribution des individus ne résulte pas du seul effet des variations temporelles et spatiales des ressources.

Les agrégations de diapause d'arthropodes terrestres, un mystère évolutif

Définition des agrégations de diapause d'arthropodes terrestres

Les agrégations de diapause consistent au regroupement d'individus adultes de la même espèce dans des sites de repos, appelés sites d'agrégation ou parfois sites d'hivernation dans les régions tempérées (exemples dans Encadré 2). Elles représentent pour des individus habituellement solitaires un passage à la vie en groupe de plusieurs mois consécutifs, cette durée pouvant représenter jusqu'aux trois-quarts de leur durée de vie. Elles correspondent à un degré peu élaboré de socialité.

Les agrégations de diapause se produisent à une période où les individus sont en diapause (Wolda & Denlinger 1984 ; Hodek 2012). La diapause est un arrêt du développement ou de l'ontogenèse programmé génétiquement (arrêt des fonctions motrices, digestives, ralentissement des fonctions reproductrices et respiratoires) couplé à des changements physiologiques assurant la survie face aux conditions environnementales

défavorables (Danks 1987 ; Košťál 2006). Les changements de conditions environnementales (baisse de la photopériode et chute des températures dans les régions tempérées ; augmentation des températures et baisse de l'humidité dans les régions tropicales ; raréfaction de la nourriture) déclenchent l'entrée en diapause (Hodek 1971 ; Goehring & Oberhauser 2002 ; Hodek 2012).

Dans la littérature, ce type d'agrégation est souvent désigné sous le simple terme d'« agrégation » (Hagen 1962 ; Hodek 2012) ou bien d'agrégation hivernale (« overwintering aggregation », Hill Jr et al. 1976 ; Holmberg et al. 1984) car la plupart des études ont été menées dans les régions tempérées. Dans cette thèse, j'utilise le terme général « agrégation de diapause » afin de regrouper sous une même dénomination les agrégations d'arthropodes adultes liées à un état de diapause qui débute en été dans les régions tempérées (estivation et estivo-hibernation ; Hagen 1962) ou en automne/hiver dans les régions tempérées (hibernation ; Hagen 1962, Hodek 2012) ainsi que les agrégations liées à la saison sèche chez les espèces subtropicales et tropicales (e.g. Wolda & Denlinger 1984). Certaines espèces d'arthropodes appartenant aux ordres des hémiptères ou des lépidoptères par exemple forment des agrégations en état de diapause à des stades immatures (œufs ou larves) (Stamp 1980 ; Fitzgerald 1993) mais elles ne sont pas l'objet de cette thèse.

Mécanismes de formation des agrégations de diapause

Chaque année, à l'approche des périodes où les conditions environnementales deviennent défavorables pour une longue durée (plusieurs mois), des centaines voire milliers d'insectes migrent de leur site de ponte (i.e. où ils sont nés et où ils s'alimentent) vers des sites d'agrégation (e.g. Tuskes & Brower 1978). La migration entre le site de ponte et le site d'agrégation peut être plus ou moins longue en termes de distance. Certaines espèces s'agrègent à proximité de leur site de ponte (Hemptinne 1988) tandis que d'autres sont capables de migrer sur de très longues distances. Les déplacements les plus longs ont été enregistrés chez la coccinelle *Hippodamia convergens* Guérin-Ménéville (Coleoptera : Coccinellidae) ou encore chez le monarque *Danaus plexippus* (L.) (Lepidoptera : Nymphalidae), capables de parcourir jusqu'à 2000 et 4000 km, respectivement (Brower 1996 ; Hodek et al. 1993 ; Hodek 2012). L'arrivée dans les sites d'agrégation s'étale sur plusieurs jours consécutifs (Brower 1996 ; Hodek 2012). Aucun individu ne rejoint l'agrégation en dehors de cette période d'arrivée. Lorsque les conditions environnementales redeviennent favorables, les individus ayant survécu quittent les sites d'agrégation et migrent vers les sites de ponte.

La localisation des sites d'agrégation reste constante d'année en année (Brower 1996 ; Sabu et al. 2008 ; Hodek et al. 2012). Cette fidélité est remarquable pour des individus à courte durée de vie voire à durée de vie annuelle car ils parviennent à trouver les sites d'agrégation occupés les années précédentes par des individus de la même espèce (Tanaka et al. 1988 ; Brower 1996). Une autre particularité des agrégations de diapause provient du stade de développement des individus qui se rassemblent. Un grand nombre d'arthropodes passent la mauvaise saison à l'état d'œufs (majorité des hémiptères phytophages, lépidoptères) ou de larves (diptères) (Danks 1978) alors que les agrégations de diapause regroupent des adultes.

Répartition des agrégations de diapause à travers les taxa

Les agrégations de diapause des coccinelles (Coleoptera : Coccinellidae) (Majerus 1994 ; Hodek 2012) et du papillon monarque *Danaus plexippus* (L.) (Lepidoptera : Nymphalidae) (Malcolm & Zalucki 1993) ont été les plus étudiées, sans doute du fait du caractère exceptionnel des migrations qui conduisent ces organismes vers leurs sites de rassemblements. Néanmoins, les agrégations de diapause sont observées pour d'autres espèces de lépidoptères et de coléoptères ainsi que des hémiptères (Tableau 1). Chez les opilions (Arachnides), les agrégations de diapause ont été observées chez de plusieurs espèces et semblent très répandues dans le genre *Leiobunum* (Tableau 1). Il y a fort à parier que des recherches spécifiques ne pourraient qu'enrichir cette liste.

Encadré 2 : Exemples d'agrégations pendant la diapause chez les arthropodes

De gauche à droite et de haut en bas :

Pyrrhocoris apterus (L.) (Hemiptera), *Danaus plexippus* (L.) (Lepidoptera)
Tytthaspis sedecimpunctata (L.) (Coleoptera), *Hippodamia variegata* (Goeze) (Coleoptera)



Tableau 1 Revue bibliographique des espèces connues pour former des agrégations de diapause à l'état adulte (agrégations liées à la saisonnalité formées à des localités constantes d'année en année)

Ordre	Espèces	Aires géographiques	Références
Coléoptère	<i>Orthotomicus erosus</i> (Wollaston)	Israël	Mendel 1983
	<i>Luprops tristis</i> Fabricius	Inde	Sabu et al. 2008
	<i>Stenotarsus rotundus</i> (Arrow)	Panama	Wolda & Denlinger 1984 ; Yoder et al. 1992
	<i>Stenotarsus subtilis</i> Arrow	Panama	Roubik & Skelley 2001
	<i>Coccinelles</i> (min. 50 espèces dans 3 familles)	Europe, Asie, Amérique, Afrique, Océanie	Révision dans le chapitre 1
Hémiptère	<i>Boisea rubrolineatus</i> (Barber)	Etats-Unis	Schowalter, 1986
	<i>Encosternum delegorguei</i> Spinola	Afrique du Sud, Zimbabwe	Dzerefos et al. 2009
	<i>Halyomorpha halys</i> Stål	Chine, Corée, Japon	Toyama et al. 2006
	<i>Lampromicra sentor</i> (Fabricius)	Australie	Monteith 1982
	<i>Leptocorisa acuta</i> (Thunberg)	Australie	Monteith 1982
	<i>Leptoglossus occidentalis</i> Heidemann	Canada	Blatt 1994
	<i>Megacopta cribraria</i> (F.)	Etats-Unis	Suiter et al. 2010 ; Golec & Hu 2015
	<i>Menida scotti</i> Puton	Japon	Koshiyama et al. 1994
	<i>Notobitus meleagris</i> Fabricius	Japon	Miyatake 1995
	<i>Odontopus sexpunctatus</i> Castelnau	Nigeria	Entwistle 1968
<i>Pyrrochoris apterus</i> L.	Europe	Socha 1993	
Lépidoptère	<i>Danaus plexippus</i> (L.)	Amérique du Nord	Malcolm & Zalucki 1993
	<i>Danaus genutia</i> (Cramer)	Taiwan	Wang & Emmel 1990
	<i>Ideopsis similis</i> L.	Taiwan	Wang & Emmel 1990
	<i>Euploea core corinna</i> (MacLeay)	Australie	Kitching & Zalucki 1981
	<i>Ithomiinae</i> sp	Brésil	Pinheiro et al. 2008

Tableau 1 (suite)

	<i>Euploea sylvester</i> (Fabricius)	Australie	Kitching & Zalucki 1981
	<i>Euploea tulliolus</i> (Fabricius)	Australie	Kitching & Zalucki 1981
	<i>Danaus hamatus</i> (Fabricius)	Australie	Kitching & Zalucki 1981
	<i>Danaus affinis</i> (Fabricius)	Australie	Kitching & Zalucki 1981
Opiliones	<i>Amilenus aurantiacus</i> Simon	Europe	Pinto da rocha et al. 2007
	<i>Gyas annulatus</i> (Olivier)	Europe de l'est	Pinto da rocha et al. 2007
	<i>Leiobunum cactorum</i> Roewer	Mexique	Wagner 1954
	<i>Leiobunum desertum</i> Goodnight and Goodnight	Mexique	Wagner 1954
	<i>Leiobunum paessleri</i> (Roewer)	Mexique	Holmberg et al. 1984
	<i>Prionostemma wagneri</i> Goodnight and Goodnight	Mexique	Wagner 1954
	<i>Serracutisoma proximum</i> Mello-Leitão	Brésil	Chelini et al. 2012

Evolution des agrégations de diapause d'arthropodes : état des connaissances

Les agrégations de diapause ont été principalement étudiées sous l'angle d'une approche fonctionnelle, selon laquelle les individus ont été sélectionnés pour vivre en groupe à une époque donnée (i.e. la finalité est de retrouver d'autres individus pour vivre en groupe) et la fonction de l'agrégation est d'augmenter la probabilité de survie des individus (Danchin & Wagner 1997 ; Danchin et al. 2008). En conséquence, la littérature est composée principalement d'études destinées à mettre en évidence des bénéfices potentiels liés à la survie pour tenter d'identifier le rôle des agrégations (paragraphes « Cause ultime n°1 » et « Cause ultime n°2 » présentés ci-après).

Cause ultime n°1 : Protection contre les ennemis naturels

La prédation constitue une pression de sélection majeure, longtemps considérée comme la cause primaire de l'évolution de la vie en groupe (Danchin et al. 2008). De nombreux animaux vivent en groupe et la grégarité s'est avérée leur fournir une protection face aux prédateurs. C'est le cas des larves d'anoures (DeVito 2003 ; Smith & Awan 2009), de divers poissons (Krause & Godin 1995), d'invertébrés (Clark & Faeth 1997 ; Uetz et al. 2002 ; Lemos et al. 2005), de petits mammifères (Hass & Valenzuela 2002 ; Rogovin et al. 2004), ou encore d'ongulés (Mooring & Hart 1992), et bien d'autres. La protection face aux prédateurs repose sur des mécanismes tels que l'effet de dilution, la vigilance accrue, l'effet de confusion ou encore la défense active en groupe (Krause & Ruxton 2002 ; Danchin et al. 2008). Par analogie, les agrégations de diapause ont été considérées comme une stratégie de défense face aux prédateurs (Hodek 1973 ; Monteith 1982 ; Holmberg et al. 1984 ; Majerus 1994 ; Vulinec 1990), selon les mécanismes cités précédemment. Cette conception est renforcée par le fait que plusieurs espèces formant des agrégations sont aposématiques, c'est-à-dire qu'elles présentent des signaux visuels remarquables associés à des moyens de défense tels que des substances olfactives repoussantes, un goût désagréable et/ou un caractère toxique (Pasteels et al. 1983).

Généralement, la grégarité chez les individus aposématiques diminue les taux d'attaque à leur encontre (Gagliardo & Guilford 1993 ; Gamberale & Tullberg 1996 ; Gamberale & Tullberg 1998 ; Riipi et al. 2001) car les groupes enverraient un signal renforcé aux prédateurs (Pasteels et al. 1983). Néanmoins, dans le contexte des agrégations de diapause, des taux de prédation plus élevés ont été enregistrés sur des individus agrégés par

rapport à des individus solitaires (Brower et al. 1977 ; Svádová et al. 2014). Par exemple, les monarques *D. plexippus* agrégés souffrent d'une prédation accrue en hiver, du fait d'oiseaux ou de petits mammifères affamés (Brower et al. 1977 ; Calvert et al. 1979 ; Glendinning & Brower 1990). Les attaques sont dirigées majoritairement contre les papillons en groupe et très rarement contre les individus solitaires (Brower et al. 1977). Seuls les individus au centre des agrégations échappent à la prédation (Brower et al. 1985). De même, chez le gendarme *Pyrochoris apterus* L. (Hemiptera : Pyrrhocoridae), les taux d'attaque sont réduits lorsque les individus sont en groupe (Svádová et al. 2014). Néanmoins, ce bénéfice semble lié au contexte géographique et à la communauté de prédateurs (Exnerová et al. 2003).

Chez plusieurs espèces de coccinelles, les agrégations hivernales se forment en réponse à des signaux volatiles, délivrés par des molécules classiquement utilisées pour la défense (Susset et al. 2013 ; Wheeler & Cardé 2013). Certains auteurs considèrent que ce mécanisme de formation renforce l'hypothèse de la défense face aux prédateurs comme fonction de l'agrégation (Wheeler et al. 2013). Néanmoins, les coccinelles disposent d'un arsenal visuel (couleurs remarquables tels que le rouge, l'orange ou le jaune) et chimique (molécules de contact telles que les alcaloïdes, Moore et al. 1990, ou molécules olfactives volatiles telles que les pyrazines, Pasteels et al. 1973) qui les protègent efficacement au cours de leur vie par leurs mécanismes de défense (Průchová et al. 2014 ; Arenas et al. 2015). Il semble peu probable que les agrégations de diapause aient évolué en tant que stratégie de défense contre les prédateurs en ce qui les concerne. Ces faits remettent en cause l'hypothèse de protection contre les prédateurs.

Par ailleurs, la transmission de maladies, de parasites et de pathogènes est un coût majeur lié à la vie en groupe (Mráček & Bečvár 2000 ; Krause & Ruxton 2002). Les agrégations de diapause sont également touchées par cette menace. Chez le monarque, Bradley & Altizer (2005) ont suggéré que la migration sur une longue distance entre les sites de ponte et les sites d'agrégation agit comme un filtre environnemental sur les individus infectés, diminuant ainsi la prévalence des parasites dans les sites d'hivernation sans l'éradiquer. Cependant, les taux de papillons infectés restent très élevés dans les sites d'hivernation : entre 53 et 68% de papillons infectés par des protozoaires dans deux populations hivernantes (Leong et al. 1992). La présence d'une faible proportion de papillons infectés dans les sites d'hivernation suffit à ce que les parasites se propagent (Altizer et al. 2000). Chez les coccinelles agrégées, la présence de champignons ectoparasites tels que *Beauveria bassiana* (Balsamo) Vuillemin (Ascomycota : Hypocreales) et *Hesperomyces virescens* Thaxter (Ascomycota : Laboulbeniales) dans les agrégations hivernales entraînent la

mort des individus dans des proportions élevées, parfois jusqu'à 80% (Nalepa & Weir 2007 ; Güven et al. 2015). Les agrégations ne semblent donc pas être une stratégie pour se protéger face aux ennemis naturels.

Cause ultime n°2 : Stratégie pour survivre face aux conditions environnementales

Les sites d'agrégation sont généralement dépourvus de ressources alimentaires. De plus, les individus qui s'y rassemblent sont en diapause, ne se nourrissent pas et ne cherchent pas à s'alimenter. Pour cette raison, il a été privilégié que les agrégations se forment soit dans des endroits propices à la survie des individus en termes de conditions abiotiques (Moseley & Hebda 2001 ; Dudek et al. 2015), soit dans des sites où malgré les conditions abiotiques défavorables les individus densément serrés les uns contre les autres créeraient un microclimat favorable (Benton & Crump 1979 ; Majerus 1994 ; Danks 2002 ; Brower et al. 2008). Une question légitime se pose rapidement : qu'est-ce qu'un site propice à la survie d'un arthropode en termes de conditions abiotiques ? Les arthropodes passent la mauvaise saison (saison sèche, hiver) en état de diapause (Danks 1987). Cet état associé à des changements physiologiques, métaboliques et comportementaux leur permet de survivre dans des conditions environnementales sévères (privation de nourriture, conditions abiotiques extrêmes, ...). Les arthropodes sont ainsi capables de s'acclimater rapidement à des températures très basses (Lee Jr & Denlinger 1985 ; Denlinger 1991 ; Lee Jr & Denlinger 1991 ; Larsen & Lee Jr 1994). Par exemple, chez le gendarme *P. apterus*, les individus non diapausants ne sont pas capables de survivre à des températures négatives alors que les individus ont un point de superfusion de -7°C lorsque la diapause commence à être induite et de -12°C lorsque la diapause est complète (Hodkova & Hodek 1997).

De manière générale, l'agrégation permet une réduction des pertes d'eau, la lutte contre la dessiccation et une réduction de la consommation d'énergie (Tanaka et al. 1988 ; Yoder et al. 1992 ; Klok & Chown 1999 ; Brower et al. 2008 ; Broly et al. 2013). En revanche, peu de données montrent que les agrégations se forment dans des endroits favorables aux arthropodes en termes de conditions abiotiques ou bien que l'agrégation fournit un microclimat approprié pour leur survie. Chez le papillon monarque, les individus hivernent dans des sites tempérés et avec de faibles précipitations, ce qui correspond à des conditions optimales pour leur survie (Oberhauser & Peterson 2003 ; Brower et al. 2008). Au sein de leurs sites d'agrégation situés dans les forêts d'*Abies religiosa* (Kunth) (Pinaceae) du Mexique et du sud des Etats-Unis, ils ont tendance à s'agréger sur les troncs à une hauteur où

les températures restent stables et chaudes pendant la nuit (Brower et al. 2011) car d'après Goehring & Oberhauser (2002), le monarque n'est pas capable de survivre au froid et notamment aux températures négatives. Chez les coccinelles, de grandes tendances généralistes ont été documentées : certaines espèces vont choisir des sites très humides tandis que d'autres auront tendance à les fuir (revues dans Hagen 1962 et Hodek 2012).

Les conditions abiotiques dans les microsites ont été enregistrées le papillon monarque *D. plexippus* (Chaplin & Wells 1982 ; Brower et al. 2008). Si l'agrégation de diapause fournit un microclimat qui limite les variations quotidiennes de température et d'humidité chez le monarque, cette variation est ténue : moins de 1°C et de 1% d'humidité de différences entre l'intérieur et l'extérieur de l'agrégation (Brower et al. 2008). Des enregistrements de température et d'humidité ont également été réalisés chez la coccinelle *Coleomegilla maculata* (DeGeer) (Coleoptera : Coccinellidae) mais aucune précision n'est donnée sur la position des capteurs de températures par rapport à l'agrégation ni si les mesures ont été réalisées au sein de l'agrégation (Benton & Crump 1979). Chez ces deux espèces, les conditions abiotiques n'ont été mesurées que dans un seul site (Benton & Crump 1979 ; Brower et al. 2008). Ainsi, les données existantes ne permettent pas de dire les conditions abiotiques dans les sites d'agrégation sont favorables à la survie des individus et si les insectes sont dans ces sites car ils recherchent des conditions abiotiques particulières.

Limites de l'approche fonctionnelle pour expliquer l'évolution des agrégations de diapause
Jusqu'à présent, il était considéré que les agrégations existent car les individus ont été sélectionnés pour vivre en groupe, au moins temporairement, et que l'agrégation permet d'améliorer leurs chances de survie. Or, comme nous venons de le voir, les bénéfices liés à la survie restent sporadiques alors que les coûts sont très répandus. Ce constat ne permet pas de formuler une explication générale à l'évolution des agrégations hivernales chez les arthropodes.

Un autre problème quant à l'application de cette approche pour l'évolution des agrégations de diapause est qu'elle ne prend pas en compte le rôle dans la reproduction que pourraient avoir les agrégations de diapause. Or, des accouplements ont été observés dans le cadre des agrégations de diapause chez plusieurs espèces. Parmi les espèces présentées dans le Tableau 1, c'est le cas de *D. plexippus* (Hill et al. 1976 ; Tuskes & Brower 1978), d'*O. erosus* (Mendel 1983), de plusieurs espèces de Nymphalidae à Taïwan (Wang & Emmel 1990), de *M. cribraria* (Golec & Hu 2015), de plusieurs espèces d'opilions (Holmberg et al.

1984), de *S. rotundus* (Wolda & Denlinger 1984), ou encore de *M. scotti* (Koshiyama et al. 1994).

Face à ce constat, il est possible que le cheminement traditionnellement pris pour comprendre l'évolution des agrégations de diapause ne soit pas adéquat.

L'hypothèse de la sélection des commodités : des colonies d'oiseaux aux agrégations hivernales d'arthropodes

Origine et principe de l'hypothèse de la sélection des commodités

Danchin & Wagner (1997) s'étonnaient que les causes évolutives sous-jacentes à la formation des colonies reproductives restent un mystère. Les colonies ont été étudiées pendant des décennies sous l'angle de l'approche fonctionnelle (Wittenberg & Hunt 1985 ; Brown & Bomberger Brown 1996 ; Rolland et al. 1998 ; Danchin et al. 2008). Or, à l'instar des agrégations de diapause des arthropodes, l'étude des colonies sous l'angle de l'approche fonctionnelle pose le problème de la multiplicité des coûts face à la rareté de bénéfices documentés, conduisant à un déséquilibre de la balance coûts-bénéfices (Brown 1988 ; Brown & Bomberger Brown 1986 ; Rolland et al. 1998 ; Varela et al. 2007). Voulant se détacher de l'approche fonctionnelle, les auteurs proposèrent l'hypothèse de la sélection des commodités en utilisant comme modèles les colonies reproductives d'oiseaux (Wagner 1997 ; Danchin & Wagner 1997). Selon cette hypothèse, l'agrégation n'est pas une finalité en soi mais le sous-produit de la sélection individuelle de ressources nécessaires pour la survie et/ou la reproduction. L'agrégation résulterait de la convergence des individus vers les zones où les ressources, encore appelées commodités, sont disponibles. Deux variantes à l'hypothèse de la sélection des commodités ont été proposées, en référence aux commodités recherchées par les individus et aux pressions de sélection impliquées. Lorsque les commodités sont les sites de ponte ou un habitat de repos, on parle d'« hypothèse de l'imitation de l'habitat », (Wagner et al. 2000), ou lorsque les commodités sont les partenaires sexuels on parle d'« hypothèse du lek caché » (Wagner 1993 ; Wagner 1997). La sélection opère non pas sur la capacité des individus à vivre en groupe mais sur leurs capacités à trouver une commodité qui les conduit à fréquenter les mêmes lieux et, en conséquence, à se retrouver en groupe.

Bien que développée pour des vertébrés, cette hypothèse offre un nouvel angle pour étudier l'évolution de la formation de groupes temporaires chez l'ensemble des animaux solitaires ou peu sociaux pour qui l'agrégation temporaire entraîne à première vue davantage de coûts que de bénéfices. Elle nous conduit à nous interroger sur d'autres pressions de

sélection qui conduisent les individus à se trouver en groupe. Le second avantage de cette hypothèse est qu'elle laisse la possibilité que la sélection sexuelle soit le moteur de la formation de groupes animaux. Enfin, elle n'exclut pas qu'il puisse y avoir d'autres bénéfices à l'agrégation mais implique que ces bénéfices (protection face aux prédateurs, par exemple) aient aidé au maintien plutôt qu'à l'apparition des agrégations.

Les agrégations d'arthropodes pendant la diapause = des leks cachés ?

La sélection sexuelle en tant que moteur des agrégations de diapause a été largement défavorisée par rapport à la sélection naturelle dans le contexte des agrégations de diapause. Bien que des accouplements aient été observés dans les sites d'agrégations, très peu d'auteurs ont émis des hypothèses faisant le lien entre les agrégations de diapause et la reproduction : on trouve, par exemple, Holmberg et al. (1984) pour expliquer le comportement d'agrégation des opilions dans les caves et les mines au Canada, par Wells & Wells (1992) pour les agrégations de monarques aux Etats-Unis et au Mexique, ou encore Hagen (1962), Majerus (1994), Iperiti (1999) au sujet des agrégations de coccinelles.

Holmberg et al. (1984) avancèrent l'idée que les agrégations chez les opilions amélioreraient le succès reproducteur. Ils réfutèrent cependant l'idée d'un lien entre agrégations et reproduction chez les opilions en argumentant que les accouplements pourraient avoir lieu lors de l'arrivée des opilions dans les caves sans contrainte de rester par la suite pendant de longs mois (Holmberg et al. 1984). Cependant, il est possible qu'au moment de leur arrivée les opilions soient en diapause (Lipovšek et al. 2002) et que les femelles refusent tout accouplement mais cela ne fut jamais vérifié. Chez le monarque, des accouplements ont été observés dans les sites d'agrégation (Hill et al. 1976) mais les travaux sur le rôle de la reproduction dans la formation des agrégations restent des travaux de modélisation (Wells et al. 1990). Majerus (1994) et Iperiti (1999) émirent l'hypothèse que les coccinelles pourraient bénéficier d'être à proximité d'individus du sexe opposé dans les agrégations de diapause pour s'accoupler avant de disperser. Néanmoins, ces auteurs ne précisent pas s'ils voient la sélection sexuelle comme moteur de la formation des agrégations de diapause.

Chez l'ensemble de ces modèles biologiques, la proportion de ces accouplements et la qualité des partenaires sexuels n'ont jamais été étudiées, ne permettant ainsi pas de savoir si les accouplements sont opportunistes ni de confirmer le potentiel rôle de la sélection sexuelle dans la formation des agrégations de diapause.

Objectifs et contenu de la thèse

Questions abordées

L'objectif de cette thèse est d'identifier les causes ultimes à l'origine de la formation des agrégations de diapause en étudiant plus particulièrement le rôle de la reproduction dans le cadre de l'hypothèse du lek caché, en utilisant comme modèle biologique la coccinelle *Hippodamia undecimnotata* (Schneider).

L'originalité de cette thèse repose sur : 1) le changement du paradigme au sujet des raisons de la formation des agrégations de diapause, 2) la mise en avant du rôle des agrégations de diapause comme stratégie de reproduction, 3) l'implication de la sélection sexuelle comme processus évolutif de la formation des agrégations de diapause.

Dans cette thèse, 4 questions principales sont posées :

- quelles sont les caractéristiques des sites d'agrégation aux échelles locale et paysagère ? (**chapitre 1**)
- les coccinelles s'agrègent-elles dans les sites qui leur offrent des conditions abiotiques favorables de survie ? (**chapitre 2**)
- la formation des agrégations hivernales est-elle une stratégie pour trouver des partenaires sexuels et s'accoupler ? (**chapitre 3**)
- quels sont les coûts énergétiques liés aux accouplements dans un contexte de privation de nourriture ? Ces coûts sont-ils un frein pour les comportements post-agrégation ? (**chapitre 4**)

Le premier chapitre est destiné à améliorer les connaissances sur les lieux où se regroupent les coccinelles. Au début de la thèse, il m'a été nécessaire de trouver des sites d'étude en région Midi-Pyrénées car les agrégations de *H. undecimnotata* ont seulement été étudiées dans d'autres pays d'Europe et dans le Sud-est de la France (Ipert 1966 ; Hodek 2012). Les modèles de distribution d'espèces (Species distribution models, SDM) sont couramment utilisés pour prédire la distribution spatiale d'une espèce. Le SDM est un modèle statistique qui relie les données de distribution d'espèces (données d'occurrence ou d'abondance à des localisations connues) avec des informations sur les caractéristiques spatiales et environnementales de ces localisations (Elith & Leathwick 2009). Dans ce chapitre, j'ai utilisé cet outil afin de déterminer la distribution des sites d'agrégation d'*H. undecimnotata* et en identifier les caractéristiques.

Etant donné que nous ne pouvons pas exclure que les coccinelles convergent vers des sites qui leur assurent des conditions abiotiques optimales de survie, j'ai dédié un court chapitre à l'étude des conditions abiotiques dans les microsites. Les caractéristiques physiques des sites d'agrégations, en partie documentées dans la littérature, sont maintenant connues grâce au chapitre 1 ; en revanche, les conditions abiotiques dans les sites d'agrégation n'ont pas été étudiées. Il est souvent admis que pour survivre les insectes se regroupent pour conserver la chaleur mais c'est une vision très vertébrée-centrée. Les connaissances physiologiques des insectes montrent au contraire que le froid n'est pas un ennemi et qu'un hiver froid et sec est meilleur pour la survie qu'un hiver doux et humide. J'ai donc mesuré la température et l'humidité relative au plus près des agrégations et j'ai analysé ces données en essayant de comprendre leur impact sur l'économie d'énergie et la prolifération de champignons ectoparasites pathogènes.

Cette thèse se poursuit par un chapitre dans lequel sont présentés les résultats de l'étude du comportement reproductif des coccinelles et de la mise en évidence des accouplements dans les sites d'agrégation. Le but est de savoir si les agrégations hivernales font partie du système de reproduction d'*H. undecimnotata* en étudiant plus particulièrement la proportion d'individus qui s'accouplent dans les sites d'agrégation et à quel(s) moment(s) ces accouplements ont lieu. Ainsi, j'ai évalué si la sélection sexuelle peut être considérée comme moteur de la formation des agrégations chez cette espèce.

Le quatrième chapitre traite de la physiologie des coccinelles pendant la période où elles sont agrégées. Les accouplements sont des événements coûteux en énergie chez plusieurs espèces. Dans le cas des agrégations hivernales, ces coûts peuvent être d'autant plus importants que les individus sont privés de nourriture pendant des mois. Dans ce chapitre, j'ai étudié si la période d'accouplements entraîne une dépense d'énergie supplémentaire pour les individus. D'autre part, je me suis intéressée à quantifier le budget énergétique au moment de la dispersion des individus pour savoir s'ils sont en mesure d'effectuer les comportements post agrégation : vol retour vers les sites de ponte, production de gamètes, ponte.

Modèle biologique et principaux sites d'étude

Cette partie présente brièvement les caractéristiques biologiques et comportementales des coccinelles (pages 30-31) et du modèle biologique, la coccinelle *Hippodamia undecimnotata* (Schneider 1792) (pages 32-35), étudié dans cette thèse ainsi que l'évolution de sa répartition en France depuis la fin du XVIIIème siècle à nos jours (page 35-50).

Une page de description des principaux sites où été menés les expériences et les échantillonnages est également proposée (page 51)

Quelques mots de présentation sur la famille des Coccinelles

Les coccinelles sont des insectes holométaboles dont le cycle de vie comprend 4 stades larvaires, un stade nymphal et un stade adulte (Figure 1). Un grand nombre d'espèces de coccinelles se nourrit de pucerons, une ressource éphémère exploitée par des guildes très diversifiées (Dixon 2000 ; Honěk & Hodek 2012). Les pucerons forment des colonies qui durent autant de temps qu'il faut aux larves de coccinelles pour se développer et atteindre le stade adulte (Dixon 2000). Les coccinelles femelles qui cherchent des colonies de pucerons pour pondre leurs œufs évaluent la qualité de celles-ci en se basant sur la présence des molécules infochimiques que laissent des congénères (Dolumbia et al. 1998). La présence de ces molécules indique que la colonie de pucerons est déjà exploitée et qu'il y a risque de compétition, de cannibalisme et/ou de pénurie alimentaire (Dolumbia et al. 1998). En conséquence, les coccinelles s'évitent et sont des animaux solitaires pendant la période de ponte.

Les agrégations de diapause se forment quelques semaines après le passage au stade adulte et durent jusqu'à la période de ponte suivante, au moins dans le cas le plus commun des espèces univoltines² (Hagen 1962 ; Hodek 2012) (Figure 1). Les coccinelles ont été beaucoup étudiées d'un point de vue naturaliste, ce qui en fait l'un des groupes les mieux connus (Hagen 1962 ; Majerus 1994 ; Hodek et al. 2012 ; Ceryngier 2015). Plus de 50 espèces de coccinelles (Coleoptera : Coccinellidae) forment des agrégations pendant la diapause (Chapitre 1). Les mécanismes de la sélection primaire des sites d'agrégation ne sont pas bien connus. En revanche, nous savons que la localisation de ces sites d'année en année est un processus multi-étapes : 1) les individus s'orientent vers les sites d'agrégation grâce à des signaux visuels (Nalepa et al. 2000 ; Nalepa et al. 2005), 2) une fois arrivés, ils répondent à des signaux chimiques volatiles et de contact pour retrouver leur congénères et se regrouper (Durieux et al. 2012 ; Susset et al. 2013 ; Wheeler et al. 2013). Pourquoi certains sites sont-ils préférés à d'autres ? Et pourquoi à l'intérieur d'un site des microsites qui semblent favorables restent-ils vides ? Ces questions restent pour le moment sans réponse.

Les coccinelles ne restent pas immobiles dans les sites d'agrégation mais modifient leurs activités en fonction des conditions météorologiques (Wheeler et al. 2013). Lors de journées ensoleillées et non venteuses les coccinelles sont plutôt dispersées alors qu'elles sont

² Espèces dont le cycle de vie comporte une seule génération par an.

étroitement serrées les unes contre les autres lorsque les conditions environnementales deviennent défavorables (pluies abondantes, forts vents, ...) (Copp 1983) (Figure 1).

Lorsque plusieurs espèces sont présentes au sein du même site d'agrégation, elles tendent à former des agrégations monospécifiques (Hodek 2012). Par exemple, *H. undecimnotata*, *Hippodamia variegata* et *Tytthaspis sedecimpunctata* sont fréquemment rencontrées dans les mêmes sites d'agrégation situés aux sommets des coteaux dans la région Midi-Pyrénées (France) mais jamais elles n'ont été observées au sein de la même agrégation (Susset, obs. pers.).

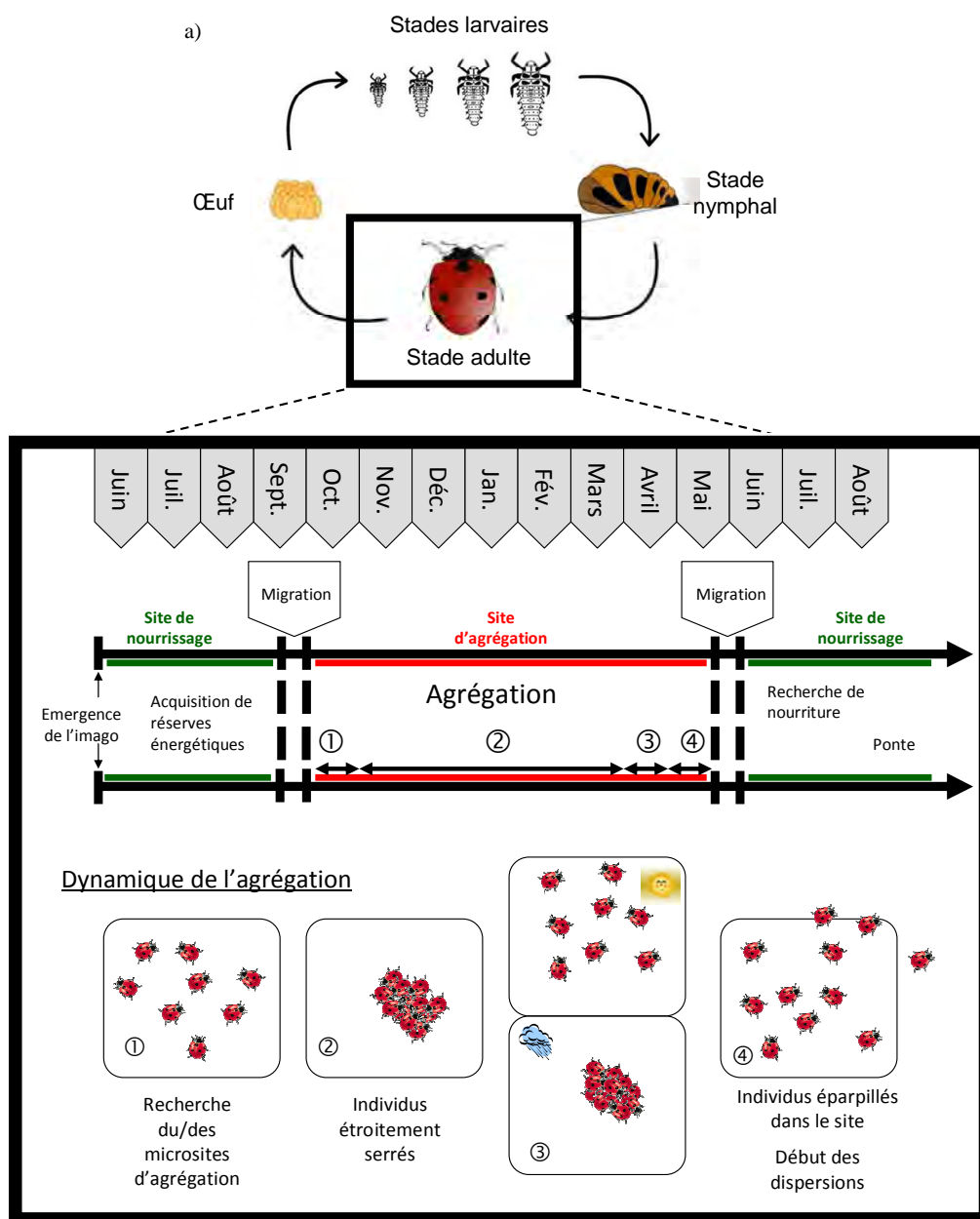


Figure 1 Chronologie de la formation des agrégations hivernales chez les coccinelles. a) Cycle de vie des coccinelles, b) Dynamique des agrégations

Le modèle biologique : *Hippodamia undecimnotata* (Schneider)

Description générale

Hippodamia undecimnotata (Schneider, 1792) est une coccinelle dont les élytres sont rouges à tâches noires (Figure A). Le nombre de tâches est variable. Sa silhouette est ovale et plus plate que la coccinelle à sept points *Coccinella septempunctata* L., une autre coccinelle rouge à points noirs avec laquelle elle est souvent confondue.



Figure A. *Hippodamia undecimnotata*
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Aire de répartition

H. undecimnotata est une espèce à distribution paléarctique (Iablokoff-Khnzorian 1982). Pour les besoins de cette thèse, des recherches ont été réalisées pour connaître sa distribution en France (p. 35). Au travers de cette étude, il apparaît que *H. undecimnotata* est présente dans la moitié sud de la France (p. 35).

Régime alimentaire et habitat de reproduction

Espèce univoltine (Hagen 1962), *H. undecimnotata* préfère pondre sur des plantes de petite taille (0-50 cm) (Iperti 1999). On la rencontre très souvent sur *Vicia faba* L. et d'autres légumineuses ainsi que de façon générale dans les strates herbacées (Iperti 1999). Ses proies sont majoritairement des pucerons des légumineuses tels qu'*Aphis fabae*, *Myzus persicae*, *Acyrtosiphon pisum* (Hemiptera) (Hodek et al. 2012). Elle peut parfois consommer les œufs du papillon *Ephestia kuehniella* (Lepidoptera : Pyralidae) en faible quantité (Hodek et al. 2012).

Prédateurs et ennemis naturels

Comme toutes les espèces de coccinelles, *H. undecimnotata* est parfois la proie d'oiseaux, de rongeurs ou d'arthropodes. Elle est vulnérable aux parasitoïdes tels que *Dinocampus coccinellae* Schrank (Hymenoptera : Braconidae) et aux champignons ectoparasites tels que *Beauveria bassiana* (Balsamo) (Ascomycota : Hypocreales) (Riddick et al. 2009).

Comportement d'agrégation de diapause

H. undecimnotata effectue une aestivo-hibernation en Europe du Sud, c'est-à-dire qu'elle entre en diapause puis gagne ses sites d'agrégation à la fin de l'été pour n'en sortir qu'au printemps de l'année suivante ; elle effectue une hibernation (entrée en diapause en automne/hiver) dans le reste de son aire de répartition (Hagen 1962 ; Katsoyannos et al. 2005). Les facteurs induisant la diapause semblent être la raréfaction de la nourriture et la diminution de la qualité de la nourriture mais les résultats restent controversés (Rolley et al. 1974 ; Iperiti & Hodek 1976). La photopériode joue également un rôle important dans la régulation de la diapause : les femelles restent en diapause lorsque la photopériode est en deçà d'un ratio de 12h de luminosité et 12h d'obscurité (Hodek & Iperiti 1983). Le rôle de la température dans l'induction de la diapause ne semble pas avoir été étudié.

Les sites d'agrégation sont généralement situés sur dans des lieux proéminents (grand rocher, amoncellement de roches, buissons, structures artificielles telles que des poteaux téléphoniques, points de triangulation, ruines, ...) (Hodek 2012 ; Chapitre 1).

Les sites d'agrégation accueillent chaque année de quelques dizaines à plusieurs milliers d'individus qui se regroupent dans des microsites étroits et restent en contact les uns avec les autres jusqu'au retour de conditions environnementales plus clémentes (Figure B). En France, on note la présence d'agrégations d'*H. undecimnotata* dès 200 m (Chapitre 1).

***Hippodamia undecimnotata* comme modèle biologique**

Les premiers travaux publiés sur *H. undecimnotata* remontent aux débuts du XX^{ème} siècle. Le célèbre entomologiste français Jean-Henri Fabre lui a consacré plusieurs passages de son œuvre phare *Souvenirs entomologiques*. Il y décrit le formidable spectacle offert par les agrégations hivernales d'*H. undecimnotata* (dont il pensait que c'était *C. septempunctata*) au sommet des monts aveyronnais. Plus récemment, le phénomène d'agrégations a été décrit par André Ferran (voir revue de ses travaux dans Coutanceau 2015) puis par deux chercheurs tchèques, Ivo Hodek et Aloïs Honek (Ceryngier 2015). Actuellement, Piotr Ceryngier continue d'étudier ses mœurs hivernales en Pologne (Ceryngier 2015).



Figure B. Agrégation hivernale d'*Hippodamia undecimnotata*
©Eline Susset

Temporal and spatial distribution of the ladybird *H. undecimnotata* (Coleoptera: Coccinellidae): insights on its range shift in France

In preparation

Introduction

Biodiversity decline is observed worldwide and affects ecosystem functioning (Worm et al. 2006; Cardinale et al. 2012). This current decline is considered as the sixth mass extinction and is mainly driven by human activities (Barnosky et al. 2011; Lewis & Maslin 2015). Recently, several researchers pointed out that insects, representatives of two-third of the total number of species (e.g. Stork 1997; Mora et al. 2011), have been neglected in studies of declines and extinctions (Dunn 2005; Conrad et al. 2006) compared to vertebrates or vascular plants (New 2004; Thomas et al. 2004). Pollinator declines in Hymenopterans have been well documented (Potts et al. 2010) because of their functional importance for plant reproduction and agricultural productivity (Ashman et al. 2004; Klein et al. 2007). By contrast, other insect species with valuable ecosystemic functions still suffer a lack of interest.

The charismatic ladybird (Coleoptera: Coccinellidae) species are one such example. Indeed, studies about the health of ladybird populations at large scales have only recently been published. They have been driven by the need to estimate the impact of the biological invasion of the Harlequin ladybird *Harmonia axyridis* (Pallas), a biological control agent that became an invader (Koch & Galvan 2008). These studies confirm native ladybird declines worldwide (USA/Canada: Harmon et al. 2007; USA: Losey et al. 2007; UK: Roy et al. 2012; Belgium: Adriaens et al. 2015). These declines manifest as range contractions or shifts, abundance decreases and even local extinction (Losey et al. 2007; Adriaens et al. 2015) caused by to intensive agriculture, habitat destruction, and competition with alien species (Wheeler & Hoebeke 1995; Harmon et al. 2007; Brown et al. 2011).

A high number of ladybird species are important predators of aphids, which are important pests in the Northern hemisphere (Hodek 2012a; Dixon 2000). Therefore, ladybirds can be an alternative to insecticides (Diehl et al. 2013). Conservation biological control (CBC) - the “modification of the environment or existing practices to protect and enhance specific natural enemies or other organisms to reduce the effect of pests” (Eilenberg et al. 2001) - seeks to maximize the abundance of beneficial insects naturally present in the landscape (Bianchi et al. 2006). There are thus increasing efforts to better integrate or manipulate these predators into agricultural practices to control aphid proliferation (e.g.

Fassotte et al. 2016; chapitre 1). However, before these species can be used as biological control agents, we require better understanding about their spatial and temporal distribution. Ladybirds depend on at least two different habitats, each with a different functional suitability: the breeding site where individuals were born, and where they feed and lay their eggs, and the aestivation or overwintering site, depending on species, where they spend the unfavourable periods (Dixon 2000; Hodek 2012b).

The ecology and biology of ladybirds have been studied for decades (reviewed in Majerus 1994 and Hodek et al. 2012), with the seven-spotted ladybird *Coccinella septempunctata* L. or the two-spotted ladybird *Adalia bipunctata* (L.) being the most studied (Sloggett, 2005). *Hippodamia undecimnotata* (Schneider) aroused the attention of the famous French entomologist, Jean-Henri Fabre, who described its overwintering habits in his seminal work a century ago (Fabre 1914). Moreover, thanks to more recent works, we know that *H. undecimnotata* ranged in Eastern and Southern Europe, including France, and in Asia (Iablokoff-Khnozorian 1982; Hodek et al. 2012). Nonetheless, its accurate spatial range in each country is far from being known. In France, no database is available for ladybirds at the country-scale, and citizen science, the involvement of volunteers in research programs to enlarge sampling effort (Bonney et al. 2009), is poorly developed. By comparison, the UK benefits from citizen science records through the Biological Records Centre since 1964 (Pocock et al. 2015), and from the UK Ladybird Survey since 1971, which cover the overall surface of the country and contain more than 15,500 records for *C. septempunctata* L. and more than 123,000 records for the two-spotted ladybird *A. bipunctata* (L.) in 2016 (<http://www.ladybird-survey.org/>). Moreover, the first atlas books about ladybirds present in France were published in 2003 and 2015 respectively; both were dedicated to ladybird communities in one French county in north-western France (Lemonier & Livory 2003; Durand & Barbier 2015), covering less than 2% of the global surface area of France.

With this in mind, the purpose of this study is to document the past and current distribution of *H. undecimnotata* in France, to investigate whether this species underwent a range shift in its breeding range across the past century, and to give insights on the current distribution of *H. undecimnotata*'s range. We chose the early years of the 1960's as a point of reference between past and contemporary data as it fairly corresponds to the beginning of the Green Revolution in France which incurred major changes in agricultural practices and landscape modifications and simplifications (Tschardt et al., 2005) and was shown to have major impacts on biodiversity (Krebs et al. 1999).

Material and methods

Studied species

H. undecimnotata is a red ladybird with black spots (Figure 1). It feeds on a wide spectrum of aphid species, breeds in herbaceous plants and overwinters at the summit of promontories such as hills and mountains (Hagen 1962; Iperiti 1966; Iperiti 1999).

H. undecimnotata has been renamed several times since its first description by D.H. Schneider in 1792. Over the centuries, it has been known as *Semiadalia undecimnotata* or *Ceratomegilla undecimnotata* (Hodek et al. 2012). Since 2013, *Ceratomegilla undecimnotata* is used again (Fauna Europae web site, consulted on February 2016) while no article has been published to explain this change in name. In addition, *H. undecimnotata* has long been confounded with *C. septempunctata* L (Hodek et al. 2012). So, some specimens identified as *C. septempunctata* are in fact *H. undecimnotata* one's and vice versa. Both species are black-spotted red ladybirds but a careful observation of their shape and the pattern of white dots make the distinction very easy.

In 2016, *H. undecimnotata*'s IUCN status is « Not evaluated ». *H. undecimnotata* is not in the Catalogue of life (even under synonymic gender names).



Figure 1. *Hippodamia undecimnotata* (commons.wikimedia.org)

Data collection: observations of H. undecimnotata during the breeding season

Data were collected between May, 2013 and February, 2016. We used extensive atlas and census data to assess the distribution of *H. undecimnotata* across France. First, we collected observations of *H. undecimnotata* in published atlas such as Lemonier & Livory 2003 and Durand & Barbier (2015). Then, we collected data from the private collections of 20 museums of natural history (Appendix 1). An email was sent to the director of entomological collections in museums to know whether there were *H. undecimnotata* specimens. We also

got in touch with 45 naturalist or entomological societies to collect data about *H. undecimnotata* presence (Appendix 1). Naturalist associations were contacted by email or by phone to collect data from their database. We also asked the director of the naturalist associations to forward our request to the association's members. The naturalist associations and the museums were located across France.

Thirdly, we visited on insect forums and used Google search engine to collect *H. undecimnotata* pictures and, when available, we collected information about location and date of the pictures. A fourth way to gather data was to check in entomological journals such as *Insectes*, *Annales de la Société française d'Entomologie*, *Ecological Entomology*, ... for search of observations of *H. undecimnotata*. Forum and entomological journals were consulted every other month from May, 2013 until February, 2016 to collect updated data. For museums' and forums' data, the identity of the ladybird (naturalized specimens or pictures) was confirmed by an entomologist with very good level of knowledge in ladybird identification or by the author to avoid misleading in the identification.

Statistical analyses

We considered data up to 1960 as “past data”, and data from 1965 until 2016 as “contemporary data”. We imposed a gap between 1960 and 1965 to provide opportunities over which range changes could occur.

Range change was investigated using a Generalized Linear Model (GLM) to analyze the effect on the latitude and longitude coordinates of past and contemporary data/the difference between mean latitude and longitude coordinates between past and contemporary data.

Statistical analyses were carried out with the R version [2.14] environment (R Development Core Team 2012).

Results

Observations of H. undecimnotata during the breeding season

We collected 284 observations of *H. undecimnotata* covering the period between 1762 and 2015. Contemporary data (from 1960 to present) represents 58% of the collected data (Figure 2). Currently, *H. undecimnotata* is known to be present with certainty in 29 counties (Figure 3). Between the past and contemporary periods, *H. undecimnotata* disappeared from 24 counties (in Northern France) while it colonize no county (Figure 2). In a large number of counties (n= 35), data are not available and do not allow knowing the current status of *H.*

undecimnotata (Figure 3). We provide information about *H. undecimnotata*'s current status county by county in Figure 3.

Past data ranged at a latitude of 45.87 ± 0.21 (mean \pm SE) and a longitude of 4.14 ± 0.23 (mean \pm SE) while contemporary observations ranged at a latitude of 44.29 ± 0.08 (mean \pm SE) and a longitude of 4.50 ± 0.19 (mean \pm SE). Contemporary observations ranged at significantly higher latitudes ($F_{1,281} = 51.15$, $P < 0.001$) but not different longitudes ($F_{1,280} = 0.42$, $P = 0.51$) than past observations, meaning *H. undecimnotata*'s range is changing southward.

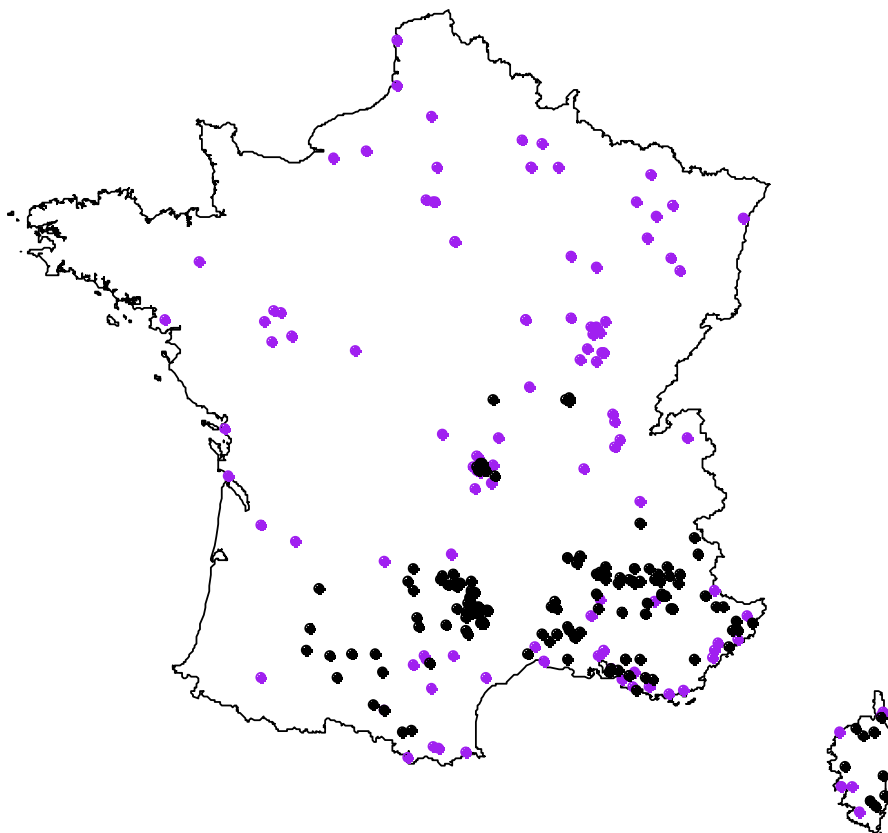


Figure 2. Past (purple dots) and contemporary (black dots) observations of the presence of *H. undecimnotata*

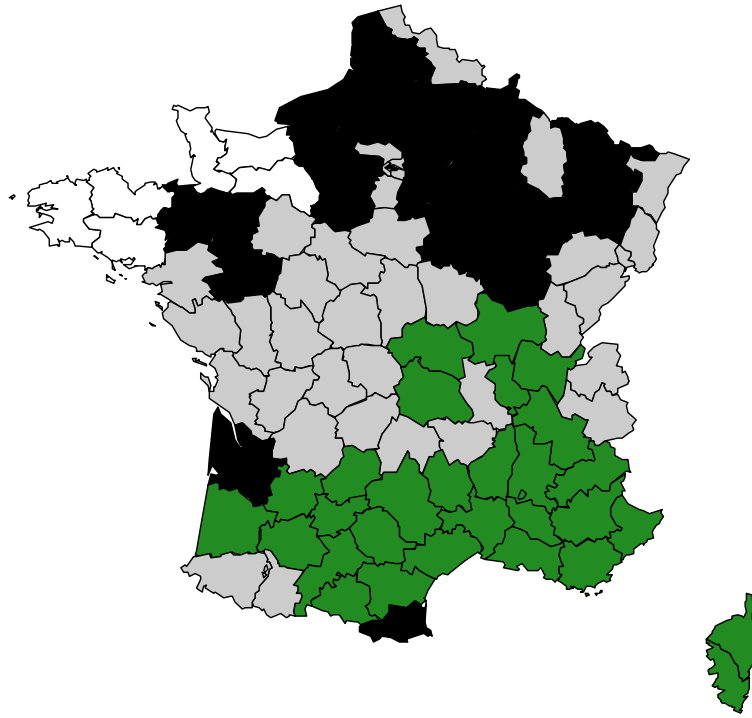


Figure 3. Current status of *H. undecimnotata* in France. White = confirmed absence ; Green = confirmed presence ; black = confirmed disappearance ; grey = no sufficient data available

Conclusion

In this study, we shed light on the current spatial distribution of the ladybird species *H. undecimnotata* in France and investigated if it changed over the past decades. As a consequence of the lack of a country-scale knowledge of ladybird distribution, we built our dataset by gathering all the data available in a wide range of sources (atlas books, naturalist websites, and museum collections) and thanks to the help of many naturalist contributors. We collected a small amount of observations, scattered throughout 4 centuries, leading us to consider our results with caution. Past distribution of *H. undecimnotata* ranged over the whole France, with exceptions in some parts close to the seaside. Our preliminary results show that since 1960's, *H. undecimnotata* has disappeared from 24 counties and has not been observed upper than N 47° latitude, indicating a change in the northern limit of its distribution while the southern limit seems stable. As no sufficient data were available for counties in the central part of France, we cannot assess with certainty the current status of *H. undecimnotata* in this geographical area.

Our preliminary results support the wide-scale patterns of range contraction observed for other ladybirds in Belgium and in the UK (Roy et al. 2012; Adriaens et al. 2015). The causes of *H. undecimnotata* range change are not known and we can only make hypotheses

about it. It may be the consequence of modernization of agricultural practices occurring during the second part of the 20th century. Iperiti (1999) indicated that chemical and physical pollution and changes in crop rotation (production of cereals instead of legumes) have detrimental impacts on *H. undecimnotata*. In the 1960s, the Green Revolution induced a simplification of the agro-systems, the destruction of semi-natural habitats and the increase of pesticide use (Tscharntke et al., 2005). All of these changes had direct negative impacts on aphids, and then an indirect negative impact for ladybirds for which aphids are the main food items (Hodek 2012a). Moreover, although pesticides do not target ladybirds, the latter are killed or are sub-lethally affected (decrease of behaviour performance, decrease of fecundity and fertility...) (Iperiti 1999; Desneux et al. 2007; Papachristos & Milonas 2008).

In addition, *Harmonia axyridis*, a ladybird introduced in Europe for biological control at the end of the 20th century, has become an invasive species that causes severe damages in local ladybird populations, and it is seen as the main agent of the rapid decline of native European ladybird species (Brown et al. 2011; Roy et al. 2012; Roy & Brown 2015). In France, impacts of *H. axyridis* on native ladybird species have not been quantified yet but its range expansion is directed from Northern to Southern France (Ternois 2012), and it is an intraguild predator of *H. undecimnotata* (Katsanis et al. 2013). Although the introduction of *H. axyridis* is more recent than the Green revolution it may accelerate *H. undecimnotata* range change and keep rendering Northern France inhospitable for *H. undecimnotata*.

Although ladybirds are cherished by the public (Majerus 1994), they have been neglected from the conservation programs and their IUCN status was assessed only recently (Adriaens et al. 2015). Among the impediments that prevent the conservation of invertebrates (Cardoso et al. 2011), *H. undecimnotata* suffers from a lack of knowledge about its distribution, abundance, and its change in space and time. Few data are available about its current range during the breeding, and even fewer on the location of its overwintering sites, which are essential places for mating and thus for the species persistence (Chapitre 3).

The consequence of the absence of long lasting data sets is the impossibility to date the onset of *H. undecimnotata* range shift and thus to infer the causes of this shift in a rigorous way. As no comprehensive samples were done during the past decades, we are unable to know why *H. undecimnotata* range changed over time and to comment on the sustainability of *H. undecimnotata*'s populations. Collecting the data about only one species was time consuming and brought a relative small amount of data compared to the amount of data collected by the Ladybird survey in UK for example. The need to assess the status of ladybird species is still

running. Citizen science can be an excellent tool to achieve this. Therefore, we highly encourage citizen science programs to be developed in France.

Appendix 1: Sources of data about *H. undecimnotata* observations

Museums contacted for the data collection

Muséum des sciences naturelles d'Angers	Muséum d'histoire naturelle du Havre
Muséum d'histoire naturelle d'Aix-en-Provence	Muséum d'histoire naturelle de Grenoble
Musée Jacques de la Comble (Autun)	Muséum d'histoire naturelle de Marseille
Muséum d'histoire naturelle d'Auxerre	Muséum d'histoire naturelle de Nantes
Muséum d'histoire naturelle de Besançon	Muséum d'histoire naturelle de Nice
Muséum d'histoire naturelle de Béziers	Muséum National d'Histoire Naturelle (Paris)
Muséum d'histoire naturelle de Bourges	Muséum d'histoire naturelle de Rouen
Musée Henri-Lecocq (Clermont-Ferrand)	Muséum d'histoire naturelle de Perpignan
Muséum d'histoire naturelle de Dijon	Muséum de Toulouse
Muséum d'histoire naturelle de La Rochelle	Muséum d'histoire naturelle de Toulon et du Var

References of collections or *H. undecimnotata*'s specimens in the museums

Museum collections

Anonyme, (MNHN)
Collection A. Argod 1931 (MNHN)
Collection A. Carret 1908 (MNHN)
Collection A. Finot 1897 (MNHN)
Collection Briel (MNHN)
Collection Charles Demaison 1937 (MNHN)
Collection de Buffévent de Muizon 1959 (MNHN)
Collection E-G Dehaut 1910 (MNHN)
Collection F. du Buysson 1900 (MNHN)
Collection G. Rouillot 1923 (MNHN)
Collection H. Coiffait 1940 (MNHN)
Collection J.C. 1907 (MNHN)
Collection L. Chopard 1922 (MNHN)
Collection Louis Bedel 1922 (MNHN)
Collection Marmottan 1914 (MNHN)
Collection Puton 1910 (MNHN)
Collection R. Peschet 1945 (MNHN)

Collection Crépin (Natural History Museum of Nice, 1969-1972)
Collection Duverger (Natural History Museum of Grenoble)
Collection V. Guédel (Natural History Museum of Grenoble)
Collection R. Duguy (Natural History Museum of La Rochelle)
Collection Henri Galibert (Natural History Museum of Toulouse)
Collection Champenois (Natural History Museum of Toulouse)
Collection Abel Félix Perrier (Natural History Museum of Toulouse)

Specimens' references

MNHN_EC_EC5920_1
MNHN_EC_EC5921_1
MNHN_EC_EC5922_1
MNHN_EC_EC5923_1
MNHN_EC_EC5924_1
MNHN_EC_EC5925_1
MNHN_EC_EC5926_1
MNHN_EC_EC5927_1

Naturalist associations

ALAST Nancy
Ariège Nature
Association Arvensis d'Expertise et de Pédagogie Naturaliste
Association Dauphinoise d'Entomologie

Association des sites du Pays Cathare
Association Lozérienne pour l'Etude et la Protection de l'Environnement
Association Roussillonnaise d'Entomologie
Charente Nature
Club Entomologique Dauphinois ROSALIA
Conservatoire des Sites Naturels de Haute-Savoie
Conservatoire Botanique National de Franche-Comté – Observatoire Régional des Invertébrés.
Conservatoire d'Espaces Naturels d'Aquitaine
Conservatoire d'Espaces Naturels de Corse
Conservatoire d'Espaces Naturels de Haute-Savoie (ASTERS)
Conservatoire d'Espaces Naturels de l'Isère
Conservatoire d'Espaces Naturels de Provence-Alpes-Côtes d'Azur
Conservatoire du Limousin
Conservatoire du Littoral
Deux-Sèvres Nature Environnement
Groupe Entomologique des Pyrénées Orientales
Groupe Ornithologique et naturaliste du Nord-Pas-de-Calais
Les Naturalistes Angevins
Les Naturalistes Vendéens
Loiret Nature Environnement
Maison de l'environnement de Franche-Comté
Manche Nature
Mayenne Nature Environnement
Nature22
Nature du Calvados
Nature du Gard
Nature et environnement en Nièvre
Nature Midi-Pyrénées
OPIE (Office Pour les Insectes et leur Environnement) Midi-Pyrénées
Picardie Nature
PNR Seine Romane
Réseau Partenarial des Acteurs du Patrimoine Naturel
Société Alsacienne d'entomologie
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Société pour l'étude et la protection de la nature en Bretagne
Société des Sciences Naturelles du Tarn-et-Garonne
Société des Sciences Naturelles Loire Forez
Société Tarnaise des Sciences Naturelles

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Les principaux sites d'étude

Mont Seigne « MS »

Altitude = 1128 m

Habitat = lande dominée par *Calluna vulgaris* Hull



Connu pour être un site d'agrégation depuis plus de 100 ans (Fabre 1914)

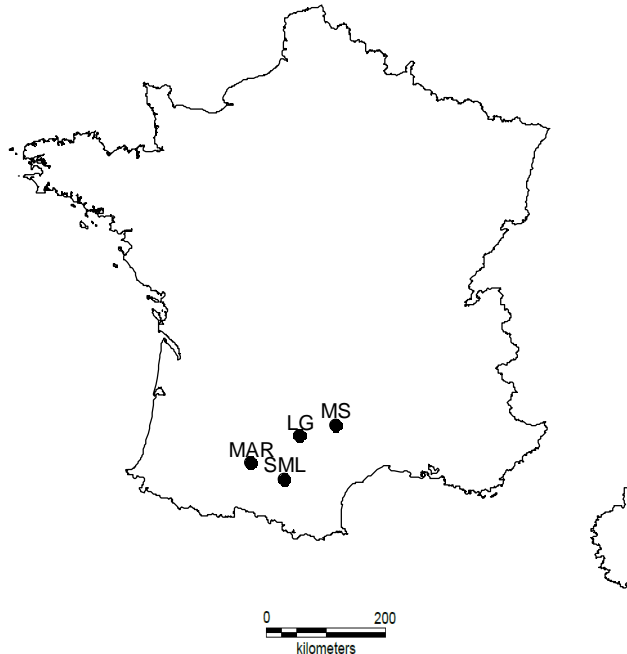
Labastide-Gabause « LG »

Altitude = 260 m

Habitat = vigne biologique entourée de cultures conventionnelles



Connu pour être un site d'agrégation depuis plus de 40 ans



Marestaing « MAR »

Altitude = 202 m

Habitat = vigne biologique entourée de cultures conventionnelles



Connu pour être un site d'agrégation depuis plus de 40 ans

Saint Michel de Lanès « SML »

Altitude = 315 m

Habitat = bande enherbée entourée de cultures conventionnelles



Connu pour être un site d'agrégation depuis 10 ans

Using Species Distribution Models to locate animal aggregations: a case study with *Hippodamia undecimnotata* (Schneider) overwintering aggregation sites

Submitted to *Ecological Entomology*



Photographie I. Exemple d'un paysage entourant un site d'agrégation
©Eline Susset

Using Species Distribution Models to locate animal aggregations: a case study with
Hippodamia undecimnotata (Schneider) overwintering aggregation sites

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1 **Abstract**

- 2 1. The protection of animals' aggregation sites is increasingly seen as a key conservation
3 strategy. However, to efficiently protect aggregation sites, we need to accurately locate
4 them. Species Distribution Models (SDMs) are an important tool in biological
5 conservation to predict spatial distribution of species and here we used them to predict the
6 distribution of the aggregation sites of a ladybird (Coleoptera: Coccinellidae) species.
- 7 2. *Hippodamia undecimnotata* forms spectacular overwintering aggregations at the same
8 locations every year across Southern and Eastern Europe. In this study, we developed a
9 SDM and tested its performance for *H. undecimnotata* aggregations in Southwest France.
10 Moreover, we looked at how environmental variables correlate with ladybirds' abundance
11 in the aggregation sites.
- 12 3. The occurrence of aggregations was best described by one model including *Isolated*
13 *prominent object*, *Pesticide risk*, *Altitude*, and *Vegetation coverage*. Furthermore, ladybird
14 abundance at the aggregation sites is positively correlated with *Altitude*. The SDM
15 occurrence model performance was found to be high (Area Under the Curve, AUC = 0.92
16 and True Skill Statistic, TSS = 0.78).
- 17 4. We suggest that *H. undecimnotata* may be an umbrella species because some aggregation
18 sites were also used by other ladybird species. Therefore, the protection of the aggregation
19 sites of this species may benefit several other species.
- 20 5. Our study provides nature conservation stakeholders with a tool to locate overwintering
21 aggregations, a first step towards the protection of these sites.

22 **Key-words:** overwintering sites, biological conservation, Coccinellidae, aphidophagous
23 insects, biological control, species distribution model.

24 **Introduction**

25 The formation, year after year, of seasonal aggregations at the same place is common across
26 the animal kingdom (Krause & Ruxton, 2002). In insects, overwintering aggregations of the
27 monarch butterfly *Danaus plexippus* L. (Lepidoptera: Nymphalidae) in the forests of Mexico
28 and Florida (Malcolm & Zalucki, 1993) are undoubtedly the most famous example of this
29 seasonal behaviour. Although less popular, the huge groups of *Leiobunum paessleri* (Roewer)
30 (Opiliones: Sclerosomatidae) in mountain forests of Canada, or of *Stenotarsus sp* Arrow
31 (Coleoptera: Endomychidae) in the tropics, are other cases of impressive aggregations
32 (Holmberg *et al.*, 1984; Wolda & Denlinger, 1984; Roubik & Skelley, 2001).

33 Ladybirds' (Coleoptera: Coccinellidae) have also raised entomologists' attention
34 because of some species' spectacular wintering aggregations. These species generally form
35 monospecific clusters of individuals densely packed from the beginning of autumn to the
36 following spring (Hodek, 2012). A review of the scarce literature on the topic indicates that,
37 among the 6000 ladybird species described worldwide (Vandenberg, 2002), a handful are
38 documented as displaying overwintering aggregations, mainly in the Coccinellinae subfamily,
39 the most studied so far (see Appendix 1 in Supplementary Material).

40 Ladybirds are cherished by children and adults and are a symbol of friendly
41 agricultural practices. Indeed, they are well known predators of pests (Obrycki *et al.*, 2009;
42 Diehl *et al.*, 2013), namely aphids (Aphididae: Hemiptera), which are among the most
43 damaging insects in temperate regions (Dedryver *et al.*, 2010). However, natural enemies of
44 pests have been negatively affected by intensive agriculture (Tschardtke *et al.*, 2005) and it is
45 increasingly accepted that ecologically based management strategies are needed to protect and
46 enhance these beneficial insects (Crowder & Harwood, 2014). In this context, the protection
47 of non-crop habitats that favour the winter survival of the natural enemies is considered
48 essential (Bianchi *et al.*, 2006). More specifically, winter aggregation behaviour displayed by

49 some ladybird species presents an opportunity to protect areas that support large numbers of
50 individuals.

51 The protection of aggregation sites is increasingly seen as a key conservation tool (e.g.
52 Hamilton *et al.*, 2011; Estrada & Arroyo, 2012; Lavers *et al.*, 2014): on the one hand,
53 aggregations occur at specific stages of animal life-history, such as mating or breeding
54 (Parrish & Edelstein-Keshet, 1999), and on the other hand the high number of individuals
55 gathered each year in the same locations makes them ideal to concentrate conservation efforts.
56 Surprisingly, the protection of ladybirds' overwintering aggregations has been neglected, and
57 little is generally known about the ecological requirements of ladybirds during winter (but see
58 reviews by Hodek (2012) and Ceryngier (2015)), an obstacle to the implementation of
59 successful conservation strategies.

60 In this work, we will focus our attention on *Hippodamia undecimnotata* (Schneider), a
61 large ladybird with red and black elytra, which is common in Southern and Eastern Europe
62 (Iablokoff, 1982). This species is one of the few that forms large overwintering aggregations,
63 made of dozens to thousands of individuals (Ceryngier, 2015). It is highly faithful to the same
64 overwintering sites: for example, overwintering sites at Mont Ventoux or Mont Seigne (South
65 of France) were already mentioned by the French entomologist J-H Fabre a century ago
66 (Fabre, 1914), and are still re-occupied every year. *H. undecimnotata* is a predator feeding
67 mainly on aphids of herbaceous plants (Iperti, 1999; Skouras *et al.*, 2015), being often
68 associated to agricultural landscapes and considered as an important beneficial insect. It has
69 therefore some attributes of a flagship species, an asset in conservation strategies (Guiney &
70 Oberhauser, 2008).

71 When forming overwintering aggregations *H. undecimnotata* displays a hypsotatic
72 behaviour, which means that it heads for prominent and isolated features on hills or
73 mountains (Hodek, 1960; Hagen, 1962; Hodek 1973; Hodek, 2012; Ceryngier, 2015).

74 Although topographic relief is an essential feature for overwintering aggregations the role
75 played by altitude is still uncertain. Indeed, aggregations occur at a wide range of altitudes,
76 going from 400 m to beyond 3000 m (Hodek, 2012; Ceryngier, 2015). Furthermore, the exact
77 nature of the support material of the overwintering microsites (the crevices in which ladybirds
78 hide and aggregate in their overwintering sites) still needs to be investigated: *H.*
79 *undecimnotata* has been seen overwintering hidden in vegetation, on rocks or artificial
80 structures but never buried in the ground (Honek *et al.*, 2007; Hodek, 2012).

81 Our work aims at characterizing the overwintering sites of *H. undecimnotata*. We will
82 seek to understand how landscape (environmental context at larger scale) and local (at
83 aggregation's scale) variables correlate with the occurrence of *H. undecimnotata*
84 overwintering aggregations. Variables related to anthropogenic activities will be included in
85 the analyses. Based on the presence/absence of aggregations and environmental variables, we
86 will develop and test a Species Distribution Model (SDM): an SDM is a model that relates
87 species distribution data (occurrence or abundance at known locations) with information on
88 the environmental and /or spatial characteristics of those locations (Elith & Leathwick, 2009).
89 Finally, we will also look at how environmental variables correlate with ladybird's abundance
90 in the aggregation sites. Our models aim at predicting the location of overwintering
91 aggregations and abundance of individuals in the aggregations to support decision-making for
92 the conservation of the overwintering habitats.

93 **Material and Methods**

94 SPECIES DISTRIBUTION MODEL BUILDING

95 *Collecting occurrence data*

96 Aggregation sites of *H. undecimnotata* were searched for in 2011 – 2013 by two
97 complementary methods: searches for potential sites on maps, and calls for witnesses (citizen
98 science, Dickinson *et al.*, 2010).

99 With the help of Ordnance Survey maps (French National Institute of Geographic and
100 Forestry information (IGN)), we defined 5 non-overlapping zones of about 500 km² each
101 corresponding to the different pedological contexts in the Midi-Pyrénées Region (South-
102 western France). Both the location and the surface area were chosen according to the
103 objectives of the work: *H. undecimnotata* is common and has been present in the Midi
104 Pyrénées region for 1 century at least (see Fabre, 1914) and the studied region corresponds to
105 an important area considering the aim of the study. Indeed, the spatial scale relevant to the
106 distributions of both species and environments in studies targeting detailed ecological
107 understanding or conservation planning tend toward local to regional extents (Elith &
108 Leathwick, 2009).

109 Because the top of hills or mountains had previously been considered as an essential
110 feature to *H. undecimnotata* overwintering aggregations (Hagen, 1962; Hodek, 2012;
111 Ceryngier, 2015), we looked for promontories in these 5 zones. We considered a promontory
112 as a raised mass of land with a drop of at least 10% relative to surrounding lowland (for
113 examples, see pictures in Hodek, 1973). Thirty promontories were identified. Field trips
114 showed that 10 of them hosted *H. undecimnotata*'s aggregations. Here we follow Hagen
115 (1962) and Honek (1989) definitions of “aggregation”: a minimum of 5 individuals touching
116 each other. In our case the smallest aggregation observed contained 10 individuals; no
117 isolated individuals were ever found. Two more promontories were occupied by *Harmonia*
118 *axyridis* (Pallas), and a third one by *Hippodamia variegata* (Goeze).

119 To increase our sampling effort, we launched the calls for witnesses. Two articles
120 were published in a local newspaper covering the Midi-Pyrénées Region, and a message was

121 broadcasted by a local radio in the same region. Readers and listeners were asked to contact
122 Eline Susset by phone or email if they had seen overwintering aggregations of ladybirds on
123 their land or knew of aggregation site locations. No further information on how or where to
124 search was given in the calls (see original texts: Delpiroux 2011a,b). So, the calls for
125 witnesses were dedicated to find aggregation sites with no a priori knowledge on *H.*
126 *undecimnotata*'s ecological preferences. Twenty-five people responded to our calls. Four
127 aggregation sites of *H. undecimnotata*, all located on promontories were identified. One of
128 these 4 sites had already been identified by our field searches and the remaining 3 were
129 outside the 5 zones we had prospected. The remaining answers concerned other species of
130 insects, including other ladybirds.

131 All the 33 sites potentially suitable for aggregations of *H. undecimnotata*, found through the
132 analysis of Ordnance Survey maps and the calls for witnesses, constitute the initial dataset on
133 the occurrence (presence/absence) of the ladybirds (Figure 1; Appendix 2).

134 *Landscape-level variables*

135 In 2013, we characterized large-scale landscape variables for each of the 33
136 promontories of the initial dataset through direct field observations and using ArcMap
137 software, ArcGis for Desktop 10.2 version ArcInfo advanced (ESRI, 2012). To characterize
138 the land use, land use polygons were digitalized and attributed on the base of georeferenced
139 numeric orthophotograph BDOrtho[®] (IGN, 2012). Seven landscape variables were considered
140 (Table 1).

141 First, the landscape variables *Crop proportion*, *Woodland proportion*, *Grassland*
142 *proportion*, *Semi-natural land proportion*, and *Urban area proportion* were calculated for 1.5
143 km circular buffer zones centred on the top of the hill of each site. *Crop proportion*
144 corresponds to the areas of arable land. Winter wheat, sunflower, maize, colza and vineyard
145 were the most common crops at each site. *Woodland proportion* corresponds to the forested

146 land, the majority of which was deciduous forest. Some landscapes also included small
147 acreages of conifer plantation as well as mixed stands of conifers and deciduous trees. We
148 treated forests as a specific landscape type and not as part of semi-natural lands since *H.*
149 *undecimnotata* mainly occurs in herbaceous vegetation (Iperti, 1999). *Grassland proportion*
150 corresponds to perennial habitats lacking dominant woody vegetation. This included old fields
151 and restored meadows, grazed pastures and forage crops. *Semi-natural Land proportion*
152 represents the hedges, the ditches, the grassy areas, the streams and wetlands. *Urban area*
153 *proportion* corresponds to residential, urban and industrialized areas.

154 Then, we evaluated two other variables for the same 1.5 km circular buffer zones: the
155 *Hedgerow density*, which is the density of linear elements within each promontory buffer
156 zone, and the *Landscape heterogeneity*, corresponding to the diversity of habitat patches
157 calculated by Simpson's reciprocal Index D:

$$158 \quad D = 1/\sum(p_i)^2$$

159 where p_i is the proportion of buffer zone covered by each land-cover category in the i^{th} land-
160 cover category (Begon *et al.*, 2006).

161 *Local variables*

162 We also considered local quantitative and qualitative variables (Table 1). Local
163 quantitative variables included *Altitude*, presence of an *Isolated prominent object*, *Vegetation*
164 *coverage*, and *Pesticide risk* (risk of pesticide spraying). *Pesticide risk* was assessed on a
165 four-levels scale ranging from 0 to 3. To do so, we first determined whether meadows or
166 crops surrounded the promontory sites. When the promontory sites were surrounded by crops,
167 we interviewed the farmers / local technical services to know if these were organic or
168 conventional crops. In the case of conventional crops we inquired if pesticides were used, and
169 the number of pesticide spraying events during the overwintering period. We also investigated
170 the presence of tracks (tramlines) left by pesticide application or pesticide residues on the

171 vegetation to complete information on pesticide events and assess if the promontory site was
172 directly sprayed by pesticides.

173 Local qualitative variables were *Habitat* (the immediate habitat context at the top of
174 the hill) and *Support* (the material of the isolated prominent object) (Table 1).

175 *Statistical analyses*

176 All the statistical analyses were carried out with the R Statistical Software version
177 3.1.1 (R Development Core Team, 2012).

178 We evaluated possible correlations between the seven quantitative landscape variables using a
179 centred and standardized Principal Component Analysis (hereafter “PCA1”) in the ade4 R
180 package (Dray & Dufour, 2007). We extracted the loadings of the 33 sites on the first three
181 PCA1 axes (PC1.1: landscape composition axis 1, PC1.2: landscape composition axis 2 and
182 PC1.3: landscape composition axis 3) that accounted for 47.5, 26.5 and 16.2% of the observed
183 variance, respectively. We used the PC1.1, PC1.2 and PC1.3 axes’ scores as explanatory
184 variables in the statistical analyses, and interpreted them as gradients for the composition of
185 the landscape surrounding each site. PC1.1 was positively correlated with *Crop proportion*
186 but negatively correlated with *Grassland proportion*, *Woodland proportion* and *Landscape*
187 *heterogeneity*. PC1.2 was positively correlated with *Semi-natural land proportion* and *Urban*
188 *area proportion*. PC1.3 was positively correlated with *Hedgerow density* (Figure 2).

189 We checked for multi-collinearity coefficients between the 4 explanatory local
190 quantitative variables by looking at the Pearson’s correlation. In cases where the correlation
191 coefficient was $> |0.7|$, only one of the variables was included in the statistical models. No
192 correlation was finally found.

193 As the site number was small (<30 ; Wisz et al 2008), the influence of both landscape
194 variables and local quantitative variables on the occurrence of aggregations was analysed

195 using Firth's penalized-likelihood logistic regression using "logistf" v1.21 R package (Heinze
196 & Ploner, 2004).

197 There were 127 candidate models from the full model to the intercept-only model.
198 Models were then compared according to the Akaike's Information Criterion, adjusted for a
199 small sample size value (AIC_c), calculated using the MuMIn v1.10.5 R package (Bartoń,
200 2009). We considered models to be different from each other only when the AIC_c difference
201 between two models was higher than 2 (Burnham & Anderson, 2002). Nagelkerke's R^2 , a
202 determination coefficient substitute for logistic regression model, was calculated for the best
203 models retained to the delta $AIC_c < 2$, following the equation:

$$204 \quad R^2 = \frac{1 - e\left(-\frac{2}{n}(LL(Full) - LL(Intercept))\right)}{1 - e\left(-\frac{2 * LL(Full)}{n}\right)}$$

205 where e is the base of the natural logarithm, $LL(Full)$ and $LL(Intercept)$ are the penalized log-
206 likelihood's associated respectively with the full and intercept-only models, and n is the
207 sample size (Nagelkerke, 1991).

208 Then, we calculated the Akaike weight of each model with the lowest AIC_c
209 (Wagenmakers & Farrell, 2004; Symonds & Moussalli, 2011). Following Wagenmakers &
210 Farrell (2004) and Symonds & Moussalli (2011) we defined the best overall model as the
211 model with the lowest AIC_c value and the highest Akaike weight value.

212 Finally, we used a Pearson's χ^2 test to compare the proportion of ladybird aggregations
213 in the different kinds of surrounding *Habitat* and *Support* material. If ladybirds do not prefer
214 any specific habitat, the expected frequencies for each kind of *Habitat* are 0.2. If ladybirds do
215 not prefer any kind of *Support*, the expected frequencies are 0.25.

216 SPECIES DISTRIBUTION MODEL PERFORMANCE

217 We tested the performance of the SDM based on a second dataset (Figure 1 and Appendix 3),
218 collected during a second field searching campaign, in 2013-2014. We selected 4 zones (15 to
219 20 km radius) where no search had been done before (Appendix 3).

220 We determined the location of 38 promontory sites potentially suitable for hosting
221 aggregations (Appendix 3) in these 4 zones using Ordnance Survey maps (IGN) and BDAI[®]
222 (IGN, 2012). All the variables previously used for the SDM described above (i.e. landscape
223 variables and local quantitative and qualitative variables) were characterized using geo-
224 referenced numeric orthophotograph BDOrtho[®] (IGN, 2012) and field visits. We used the
225 SDM retained with the first data set (model 1, Table 2) to predict the probability of
226 aggregation occurrence at each site, using R software version 3.1.1 (R Development Core
227 Team, 2012). With the “predict” R function, we calculated continuous probabilities and
228 converted them into binary predictions. The probabilities we obtained were ranged between 0
229 to 0.30 and between 0.70 to 1. Probabilities between 0 to 0.30 were converted into 0 “no
230 probability of finding an aggregation” while probabilities between 0.70 to 1 were converted
231 into 1 “high probabilities of finding an aggregation”. Finally, we investigated the top of the
232 promontory sites and surroundings to check if there were aggregations.

233 To evaluate the performance of the SDM, we calculated the Area Under the receiver
234 operating characteristic Curve (AUC) value (Hanley & McNeil, 1982). As AUC value alone
235 can lead to misleading conclusions (Lobo *et al.*, 2008), we also calculated the sensitivity (i.e.
236 probability that the model correctly classifies a presence), the specificity (i.e. probability that
237 the model correctly classifies an absence) and the True Skill Statistic (TSS) values of the
238 SDM for the second dataset (Allouche *et al.*, 2006).

239 LADYBIRD ABUNDANCE AT THE AGGREGATION SITES

240 In 2013-2014, we counted the number of ladybirds per aggregation in 20 aggregation sites
241 from both the initial and the second datasets. However, this small number of aggregations
242 prevented the building of a robust SDM (Wisz et al 2008). Therefore, we were only able to
243 perform a GLM analysis (described below) that corresponds to the first step of a SDM
244 building (Guisan & Zimmermann 2000).

245 We used a second PCA (PCA2) to investigate the correlations between landscape
246 variables at the sites. PC2.1 (landscape composition axis 1 of PCA2) was positively correlated
247 with *Grassland proportion*, *Woodland proportion*, *Urban area proportion* and negatively
248 with *Crop proportion*. PC2.2 (landscape composition axis 2 of PCA2) was positively
249 correlated with *Semi-natural land proportion*. PC2.3 (landscape composition axis 3 of PCA2)
250 was negatively correlated with *Hedgerow density*.

251 We analyzed the influence of the landscape and local quantitative variables on the
252 abundance of the ladybirds in the 20 sites using a generalized linear model, with a
253 quasipoisson family and a log link function to correct for the overdispersion of our data.
254 We checked for multicollinearity by inspecting the Pearson's correlation coefficients between
255 the 3 local variables (excluding the *Isolated Prominent Object* as all the sites in which the
256 aggregations were found had one). In cases where the correlation coefficient was $> |0.7|$, only
257 one of the variables was included in the statistical models. No correlation was finally found.

258 **Results**

259 SPECIES DISTRIBUTION MODEL BUILDING

260 *Effects of landscape and local variables on the occurrence of the overwintering aggregations*
261 *according to the SDM*

262 With regard to AIC_c, 4 models best explained the occurrence of the overwintering
263 aggregations. These 4 highest performing models include *Altitude*, *Isolated Prominent Object*,
264 and *Pesticide risk* but only 2 of these 4 models include the first axis of the first PCA (PC1.1)
265 or *Vegetation coverage* (Table 2). Using the Akaike weight criterion, Model 1 was defined as
266 the best overall model. According to this model, the overwintering aggregations have a higher
267 probability to occur when an *Isolated prominent object* is present but a lower probability
268 when *Pesticide risk* increases. *Altitude* and *Vegetation coverage* are not important explanatory
269 variables (Table 2). It is the performance of this SDM that will be tested here after.

270 The surrounding local *Habitat* of the *H. undecimnotata*'s aggregations were crops
271 (75% of the sites), woodland (17%) and grassland (8%) but never urban areas. The *Habitat*
272 had a significant influence on the occurrence of an overwintering aggregation ($\chi^2 = 21.23$, DF
273 = 4, $P < 0.001$). By contrast, the *Support* material has no influence ($\chi^2 = 5.7043$, DF = 3, $P =$
274 0.12).

275 SPECIES DISTRIBUTION MODEL PERFORMANCE

276 The second dataset consists of 38 promontory sites. According to the SDM, 16 sites were
277 characterized as "potential aggregation sites" for *H. undecimnotata*. From those potential
278 aggregation sites, the field trips indicate that 10 were effective aggregation sites. No
279 aggregation was found in the sites wherever no aggregation occurrence was predicted by the
280 SDM (Appendix 3). This leads to 84.3% of overall correct attributions by the SDM, with an
281 AUC of 0.92, and SDM sensitivity, specificity, and TSS of 1, 0.78, and 0.78, respectively.

282 LADYBIRD ABUNDANCE IN THE AGGREGATION SITES

283 Ladybird abundance ranged from 10 to up to 2500 (mean \pm SE: 286 ± 121 in 2015). It was
284 influenced by the *Altitude* ($F_1 = 35.66$, $P < 0.05$) but not by PC2.1 ($F_1 = 3.18$, $P = 0.10$),

285 PC2.2 ($F_1 = 1.85$, $P = 0.19$), PC2.3 ($F_1 = 0.04$, $P = 0.84$), *Pesticide risk* ($F_1 = 0.12$, $P = 0.73$)
286 or *Vegetation coverage* ($F_1 = 0.005$, $P = 0.94$).

287 **Discussion**

288 Species Distribution Models (SDMs) have become an important tool in biological
289 conservation to predict the spatial distribution of species at macroecological levels (reviews
290 by Guisan & Thuiller, 2005 and Elith & Leathwick, 2009). Here, for the first time to our
291 knowledge, we developed models to predict the location of aggregations sites of a ladybird
292 species. Our results provide valuable insights into the landscape and local variables that are
293 more likely to drive the distribution of aggregation sites as well as the abundance of the
294 ladybird *H. undecimnotata* per site.

295 Our SDM is robust, with 84.3% of the predicted aggregation sites correctly classified
296 and high values of AUC, sensitivity, specificity and TSS. We found that the occurrence of the
297 overwintering aggregations is best explained by the following variables: *Isolated Prominent*
298 *Object* and *Pesticide risk*. *Altitude* and *Vegetation coverage* are not important explanatory
299 variables for the occurrence of aggregations. Nevertheless, the abundance of ladybirds per
300 overwintering site is positively correlated with *Altitude*.

301 Our results thus confirm Hagen (1962) and Hodek (2012) observations that *H.*
302 *undecimnotata* preferentially aggregates at the base of isolated prominent objects. Although
303 the top of hills or mountains had previously been shown to be an essential feature to *H.*
304 *undecimnotata* overwintering aggregations (Hagen, 1962; Hodek, 2012; Ceryngier, 2015) and
305 therefore promontories were not tested in this work, we checked the area (perimeter 300 m)
306 around the top of promontories: in only one out of 38 sites was there one lower altitude *H.*
307 *undecimnotata*'s aggregation, made of a much smaller number of individuals than the top
308 aggregation. Furthermore, our results show that the altitude of these promontories is

309 positively correlated to the number of ladybirds per aggregation. This had not been shown
310 before. Overwintering at the base of isolated or visually contrasted objects located at the top
311 of hills and low mountains is a behaviour that *H. undecimnotata* shares with *Coccinella*
312 *septempunctata* L. in some parts of its range as well as *H. axyridis* (Nalepa *et al.*, 2005;
313 Hodek, 2012). These objects could work as landmarks that guide ladybirds during their
314 migration from the breeding sites to the aggregation sites, and help them to reach a
315 *rendezvous* point with conspecifics more efficiently (e.g. Majerus, 1994).

316 Our results also suggest that the overwintering aggregations of *H. undecimnotata* are
317 mainly located in agricultural crop areas and less often in grassland and forest areas. This
318 might be related to the abundance of prey available in those habitats in autumn, prior to the
319 winter period,. Moreover, aggregations of *H. undecimnotata* were never found in urban zones,
320 contrary to the invasive harlequin ladybird *H. axyridis* (Wang *et al.*, 2011). However,
321 although mainly located in agricultural areas, the overwintering aggregations of *H.*
322 *undecimnotata* occur mostly in sites with a low risk of pesticide spraying. Indeed, many
323 pesticides, including herbicides and fungicides, are toxic to beneficial insects (Desneux *et al.*,
324 2007; Papachristos & Milonas, 2008). In addition, pesticides indirectly affect beneficial
325 insects by killing their prey.

326 Of the 22 aggregation sites in this study, 6 underwent severe modifications during the
327 last four overwintering seasons, following direct or indirect anthropogenic actions. Only 1 of
328 6 sites was re-occupied on the year after, showing low site resilience. Habitat destruction and
329 fragmentation are among the most pervasive causes of biodiversity loss (Fahrig, 2003).
330 Ladybirds suffer a high proportion of natural mortality (up to 80%) in overwinter aggregations
331 (Güven *et al.*, 2015). In addition, the destruction of the aggregation sites probably forces
332 ladybirds to search further in autumn, spending more energy, and therefore incurring a higher
333 risk of mortality (Bonte *et al.*, 2011; Hein *et al.*, 2012). This adds to the many threats

334 affecting ladybird populations, such as spring habitat clearings when farmers set fire to or cut
335 the dried vegetation, and the competition with invasive alien species (Comont *et al.*, 2014).

336 This work provides a method to locate the overwintering aggregations of *H.*
337 *undecimnotata* without the need of extensive naturalist skills. This method can be proposed to
338 civil servants or stakeholders engaged in the protection of biodiversity, as a support for
339 strategic decision making in the protection of critical habitats used by large numbers of
340 ladybirds in winter. The protection of the aggregation sites of *H. undecimnotata* can have
341 cascading positive effects on the biodiversity. Indeed, we consider *H. undecimnotata* as an
342 umbrella species (Caro, 2010) because it shares some of its overwintering sites with other
343 ladybird species such as the aphidophagous *C. septempunctata* or the mycophagous
344 *Tytthaspis sedecimpunctata* (L.) and *Psyllobora vigintiduopunctata* (L.) (Susset, pers. obs.).
345 One future objective could be to evaluate the performance of this model at a broader
346 geographic scale, and for other species. Moreover, although insects are rarely considered as
347 flagship species (*sensu* Simberloff, 1998), contrarily to more emblematic vertebrates,
348 ladybirds' charisma could help raise public awareness on the importance of overwintering
349 habitats in general and on overwintering aggregations in particular, and help implementing
350 conservation strategies to protect beneficial insects all year round.

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502 Table 1. Spatial predictors used in the SDM (aggregation occurrence) and the model “Abundance”

Variable name (unit)	Variable description	Scale	Code
<u>Explained variables</u>			
Occurrence of aggregation	Presence or absence of an aggregation of <i>Hippodamia undecimnotata</i>	Binary value (0: absence; 1: presence)	
Ladybird abundance	Number of ladybirds in the overwintering aggregation in 2015	10 to > 2500.	
<u>Landscape variables</u>			
<i>Crop proportion (%)</i>	Proportion of the buffer zone covered by crops (i.e. arable lands)	0 to 89.1	Pcrop
<i>Woodland proportion (%)</i>	Proportion of the buffer zone covered by woodland (forest, warren, ...)	3.9 to 80.3	Pwood
<i>Grassland proportion (%)</i>	Proportion of the buffer zone covered by grasslands (old fields and restored prairie, grazed pastures and forage crops)	0 to 31	Pgrass
<i>Semi-natural land proportion (%)</i>	Proportion of the buffer zone covered by semi-natural habitat (the hedges, the ditches, the grassy areas, the streams and wetland areas)	0.1 to 36.6	Pnat
<i>Urban area proportion (%)</i>	Proportion of the buffer zone covered by towns, villages, roads, farm buildings, industries.	0 to 53.6	Purban
<i>Hedgerow density (m)</i>	Total linear length of hedges within the buffer zone	10,888 to 44,213	HegDen
<i>Landscape heterogeneity</i>	Simpson’s diversity reciprocal index (1/D)	1.25 to 3.18	D

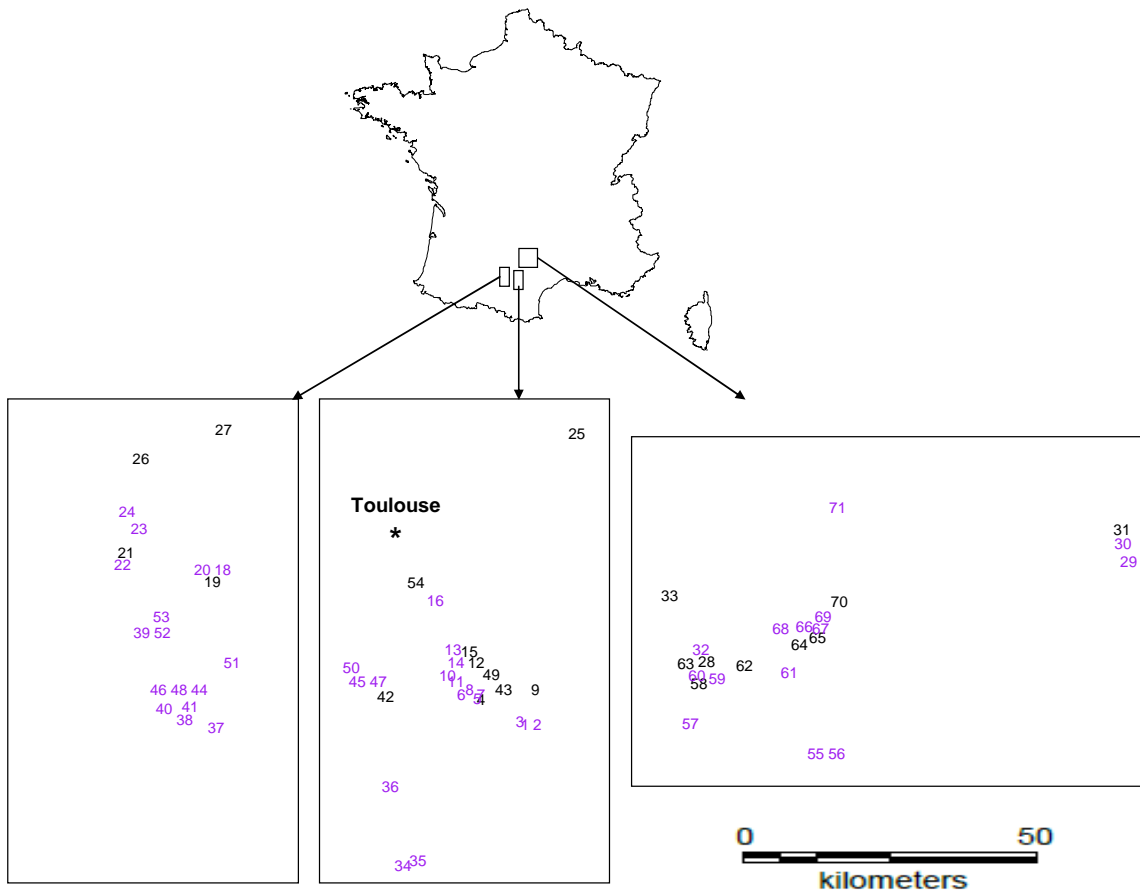
Table 1 (continued)

<u>Local quantitative variables</u>		
<i>Altitude</i>	Altitude of the highest point of the studied site (m a.s.l.)	190 to 1785 m
<i>Isolated prominent object</i>	Presence of an prominent object (i.e. height > 2 m ; width < 10 m) offering a shelter (crevice) with no surrounding relief	Binary value (0 : absence of object or absence of crevice inside the object ; 1 : presence of object with a crevice)
<i>Vegetation coverage</i>	Degree of openness intensity in a 10 m quadrat centred on the top of the hill of the studied site	1-4 : open to close (discrete variable) 1: 0-25% 2: 25%-50% 3: 50%-75% 4: 75%-100%
<i>Pesticide risk</i>	An evaluation of the risk that the highest point of the studied site be sprayed with pesticides	0 – 3 : non-existent to very high (discrete variable) 0: no risk (meadows) 1: organic crops 2: indirect spraying (wind drift); No more than 1 spraying event 3: direct spraying and /or spraying events > 1
<u>Local qualitative variables</u>		
<i>Habitat</i>	Habitat type surrounding the highest point of the studied site (radius of 100 m)	Crop, forest, grassland, urban area, semi-natural habitat
<i>Support</i>	Composition and aspect (colour) of the isolated prominent object	Vegetable (tree, vine stock, bramble), wood (phone pole, dead wood), stone (rocks, buildings), metal (electricity pole)

503 Table 2. Summary of the model selection statistics explaining the occurrence of *Hippodamia undecimnotata*'s
 504 aggregations: AICc, R², Delta AICc (Δi) and Akaike weight (W_i) are given for models with the lowest AICc values.

	Model	Estimate	Lower (95% CI)	Upper (95% CI)	p-value	AICc	R²	Δi	W_i
1	(Intercept)	0.05	-5.36	5.04	NS				
	<i>IPO</i>	3.57	1.07	8.75	< 0.01				
	<i>Altitude</i>	-0.0004	-0.005	0.007	NS	14.00	< 0.1	0.00	0.48
	<i>Veg. cover.</i>	-1.07	-3.25	0.13	NS				
	<i>Pesticide</i>	-1.11	-2.56	-0.16	< 0.05				
2	(Intercept)	-2.49	-7.41	-0.15	< 0.03				
	<i>IPO</i>	4.80	2.24	9.86	< 0.001	14.28	0.674	0.28	0.13
	<i>Altitude</i>	-0.001	-0.006	0.002	NS				
	<i>Pesticide</i>	-1.12	-2.46	-0.18	< 0.05				
3	(Intercept)	2.06	-3.32	12.26	NS				
	<i>IPO</i>	3.13	0.62	11.58	< 0.05				
	<i>Altitude</i>	-0.00001	-0.008	0.02	NS	15.37	< 0.1	1.37	0.25
	<i>Veg. cover.</i>	-1.95	-10.40	-0.20	< 0.05				
	<i>Pesticide</i>	-1.67	-7.41	-0.35	< 0.01				
	<i>PCI</i>	1.22	-0.44	7.23	NS				
4	(Intercept)	-3.49	-9.06	-0.14	< 0.05				
	<i>IPO</i>	5.22	2.22	10.73	< 0.001				
	<i>Altitude</i>	0.0005	-0.005	0.01	NS	15.48	0.863	1.48	0.13
	<i>Pesticide</i>	-1.37	-3.01	-0.25	< 0.05				
	<i>PCI</i>	0.51	-0.50	1.78	NS				

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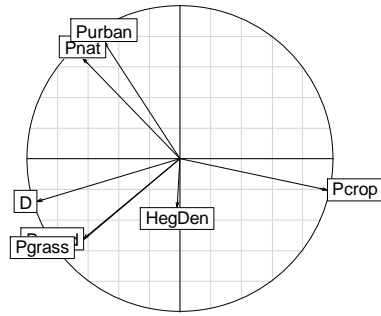


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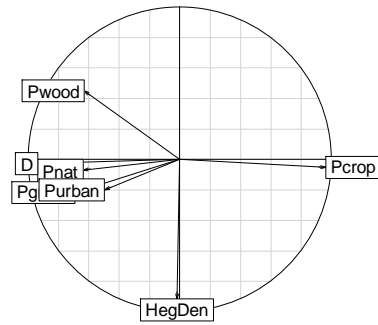
507 Fig. 1. Map of the location of the studied sites (initial and second datasets). Black number: sites with
 508 overwintering aggregations. Purple number: sites without overwintering aggregation. Sites 1 to 33: first dataset;
 509 sites 34 to 71: second dataset.

510

a)



b)



511 c)

	Axis 1	Axis 2	Axis 3
<i>Crop proportion “Pcrop”</i>	0.96	-0.20	-0.05
<i>Woodland proportion “Pwood”</i>	-0.63	-0.52	0.44
<i>Grassland proportion “Pgrass”</i>	-0.69	-0.58	-0.21
<i>Semi-natural land proportion “Pnat”</i>	-0.63	0.65	-0.06
<i>Urban area proportion “Purban”</i>	-0.49	0.76	-0.19
<i>Landscape heterogeneity “D”</i>	-0.93	-0.27	-0.02
<i>Hedgerow density “HegDen”</i>	-0.01	-0.31	-0.91

512 Fig. 2. Principal component analysis of the landscape variables potentially influencing the occurrence of
 513 *Hippodamia undecimnotata*’s aggregations. a) Correlation circle for axis 1 – axis 2, b) Correlation circle for axis
 514 1 – axis 3, c) Correlation values.

515 **SUPPLEMENTARY MATERIAL**

516 **Appendix 1.** Overwintering aggregations in the Coccinellidae: range, food preferences and group size (which may vary depending on the geographic range). (i) Food preferences are
 517 given according to Magro et al. 2010 (23), except for *Aiolacaria hexaspilota*: (4), *Micraspis frenata*: (22)

Ladybird species	Range	Food preference (i)	Size [number of individuals]			References
			Small [5-10]	Medium [10 ; 100]	Large [> 100 - ∞]	
Chilocorinae						
<i>Brumus octosignatus</i> (Gebler)	France				x	(1)
<i>Chilocorus bipustulatus</i> (L.)	Europe	Coccids	x			(2)
<i>Chilocorus renipustulatus</i> (L.G. Scriba)	Europe	Coccids	x			(2)
<i>Curinus coeruleus</i> Mulsant	Hawaiï			x		(1)
<i>Exochomus flavipes</i> Thunberg	Africa	Coccids				(3)
<i>Exochomus melanocephalus</i> (Zoubkoff)	Russia, Asia					(3)
<i>Exochomus quadripustulatus</i> (L.)	Europe	Aphids	x			(2)
<i>Platynaspis luteorubra</i> (Goeze)	Europe	Aphids		x		(1)
Coccinellinae						
<i>Adalia bipunctata</i> (L.)	Europe	Aphids		x	x	(2)
<i>Adalia decempunctata</i> (L.)	Europe	Aphids	x	x		(2)
<i>Aiolacaria hexaspilota</i> (descriptor not found)	Asia	Chrysomelids		x	x	(1) (4)
<i>Anatis ocelatta</i> (L.)	Europe	Aphids	x			(1) (2)
<i>Anisosticta novemdecimpunctata</i> (L.)	Europe	Aphids	x			(2)
<i>Calvia quatuordecimguttata</i> (L.)	Europe	Aphids	x			(1) (2)

		Larvae: aphids ;			
<i>Ceratomegilla fuscilabris</i> (Mulsant)	Europe, North America	Adults: pollen, fungi		x	(5) (6)
<i>Coccinella magnifica</i> Redtenbacher	Europe	Aphids	x		(2)
<i>Coccinella novemnotata</i> Herbst	USA, Southern Canada	Aphids		x	(7)
<i>Coccinella quinquepunctata</i> L.	Europe	Aphids	x		(1) (2)
<i>Coccinella reitteri</i> Weise	Asia	Pollen	x		(1)
<i>Coccinella repanda</i> Thunberg	Australia	Aphids		x x	(8) (9)
<i>Coccinella septempunctata</i> L.	Europe	Aphids		x x	(1) (2) (10)
<i>Coccinella transversoguttata</i> Faldermann	Canada	Aphids		x	(1) (11)
<i>Coccinella undecimpunctata</i> L.	New-Zealand	Aphids		x	(2)
<i>Coccinula cf. sinensis</i> (Weise)	Japon	Aphids	x		(1)
<i>Coccinula quatuordecimpustulata</i> (L.)	Europe	Aphids	x		(4)
<i>Coleomegilla maculata</i> (DeGeer)	North America	Aphids		x	(12)
<i>Halyzia sedecimguttata</i> (L.)	Europe	Fungi	x	x	(1) (2)
<i>Harmonia axyridis</i> (Pallas)	Asia	Aphids		x	(13) (14)
<i>Harmonia conformis</i> (Boisduval)	France, Australia	Aphids		x	(15)
<i>Harmonia quadripunctata</i> (Pontoppidan)	Europe	Aphids	x	x	(1) (2) (8)
<i>Hippodamia caseyi</i> Johnson	North America	Aphids	x		(4)
<i>Hippodamia convergens</i> (Guérin-Méneville)	North America	Aphids		x	(16)
<i>Hippodamia quinquesignata</i> (Kirby)	North America	Aphids		x x	(1) (17)
<i>Hippodamia tredecimpunctata</i> (L.)	Europe, Asia			x	(1) (8) (11)
<i>Hippodamia undecimnotata</i> (Schneider)	Europe, Asia	Aphids		x	(8) (18)

<i>Hippodamia variegata</i> (Goeze)	Algeria	Aphids	x			(1) (2) (10)
<i>Micraspis discolor</i> (Fabricius)	Asia	Aphids, leafhoppers			x	(1)
<i>Micraspis frenata</i> (Erichson)	Australia, New Caledonia	Pollen, nectar			x	(19)
<i>Micraspis furcifera</i> (Guérin-Méneville)	Australia		x			(1)
<i>Myrrha octodecimguttata</i> (L.)	Europe, Asia	Aphids	x			(1) (2) (8)
<i>Myzia oblongoguttata</i> (L.)	Europe, Asia	Aphids				(2)
<i>Oenopia conglobata</i> (L.)	Europe	Aphids	x		x	(1)
<i>Oenopia doublieri</i> (Mulsant)	Algeria	Aphids	x			(1)
<i>Oenopia guttata</i> Blackburn	Australia		x			(1)
<i>Propylea quatuordecimpunctata</i> (L.)	Europe	Aphids	x			(1) (2) (20)
<i>Psyllobora vigintiduopunctata</i> (L.)	Europe	Fungi		x	x	(1) (8)
<i>Spiladelphia barovskii</i> Semenov-Dobzhansky	Europe			x		(21)
<i>Tytthaspis sedecimpunctata</i> (L.)	Europe	Fungi		x	x	(2) (18)
<i>Vibidia duodecimguttata</i> (Poda)	Europe	Fungi		x	x	(1) (8)
Epilachninae						
<i>Epilachna bisquadripunctata</i> (Gyllenhal)	Asie	Plants			x	(22)
<i>Epilachna dregei</i> Mulsant	Africa	Plants	x			(22)

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Site number	County	Geographic coordinates		Altitude (m a.s.l)	Aggregation occurrence
		<i>Latitude</i>	<i>Longitude</i>		
1	Ariège	N 43° 15' 46.10"	E 1° 43' 41.69"	326	0
2	Ariège	N 43° 15' 48.03"	E 1° 43' 11.50"	305	0
3	Ariège	N 43° 16' 3.71"	E 1° 42' 1.16"	338	0
4	Haute-Garonne	N 43° 18' 12.99"	E 1° 36' 26"	219	1
5	Haute-Garonne	N 43° 18' 29.31"	E 1° 35' 50.12"	310	0
6	Haute-Garonne	N 43° 18' 48.54"	E 1° 34' 24.00"	296	0
7	Haute-Garonne	N 43° 19' 10.99"	E 1° 34' 56.22"	305	0
8	Haute-Garonne	N 43° 19' 19.20"	E 1° 34' 40.03"	292	0
9	Aude	N 43° 19' 25.84"	E 1° 44' 10.55"	315	1
10	Haute-Garonne	N 43° 20' 41.94"	E 1° 31' 33.70"	297	0
11	Haute-Garonne	N 43° 20' 5.12"	E 1° 32' 48.00"	302	0
12	Haute-Garonne	N 43° 22' 4.09"	E 1° 33' 48.20"	288	1
13	Haute-Garonne	N 43° 22' 59.10"	E 1° 32' 16.31"	294	0
14	Haute-Garonne	N 43° 22' 8.34"	E 1° 32' 43.82"	275	0
15	Haute-Garonne	N 43° 23' 17.05"	E 1° 34' 34.64"	265	1
16	Haute-Garonne	N 43° 28' 34.72"	E 1° 29' 37.45"	267	0
17	Haute-Garonne	N 43° 33' 48.12"	E 1° 26' 27.13"	225	0
18	Gers	N 43° 34' 42.05"	E 1° 0' 51.04"	190	0
19	Gers	N 43° 34' 54.39"	E 0° 59' 59.48"	262	1
20	Gers	N 43° 35' 3.04"	E 1° 0' 2.62"	226	0
21	Gers	N 43° 36' 47.30"	E 0° 43' 24.55"	253	1
22	Gers	N 43° 36' 56.03"	E 0° 42' 43.76"	266	0
23	Gers	N 43° 41' 50.96"	E 0° 45' 40.52"	216	0
24	Gers	N 43° 44' 8.81"	E 0° 43' 21.73"	171	0
25	Tarn	N 43° 46' 10.98"	E 1° 49' 38.73"	225	1
26	Gers	N 43° 51' 38.55"	E 0° 45' 41.90"	190	1
27	Tarn-et-Garonne	N 43° 55' 43.49"	E 1° 1' 24.61"	212	1
28	Tarn	N 44° 1' 29.11"	E 2° 6' 48.43"	260	1
29	Aveyron	N 44° 10' 12.06 "	E 2° 56' 32.66"	836	0
30	Aveyron	N 44° 12' 15.33"	E 2° 55' 50.79"	1050	0
31	Aveyron	N 44° 12' 55.95"	E 2° 55' 46.12"	1128	1
32	Tarn	N 44° 2' 31.30"	E 2° 6' 0.55"	300	0
33	Tarn	N 44° 7' 4.29"	E 2° 2' 20.99"	510	1

Site number	County	Geographic coordinates		Altitude (m a.s.l)	Model prediction	Effective aggregation occurrence
		Latitude	Longitude			
34	Ariège	N 43° 00' 44.165"	E 1° 25' 44.648"	409	0	0
35	Ariège	N 43° 01' 21.101"	E 1° 27' 48.545"	570	0	0
36	Ariège	N 43° 08' 59.39"	E 1° 23' 38.997"	389	0	0
37	Haute-Garonne	N 43° 14' 42.0"	E 1° 01' 22.0"	373	0	0
38	Haute-Garonne	N 43° 15' 42.1"	E 0° 55' 25.7"	359	0	0
39	Ariège	N 43° 16' 28.1"	E 1° 21' 19.2"	208	1	0
40	Gers	N 43° 17' 08.3"	E 0° 51' 23.3"	361	0	0
41	Haute-Garonne	N 43° 17' 34.7"	E 0° 56' 25.0"	318	0	0
42	Haute-Garonne	N 43° 18' 24.769"	E 1° 22' 47.343"	310	1	1
43	Haute-Garonne	N 43° 19' 23.025"	E 1° 39' 36.34"	286	1	1
44	Haute-Garonne	N 43° 19' 50.6"	E 0° 54' 11.0"	297	0	0
45	Haute-Garonne	N 43° 19' 56.5"	E 1° 20' 07.4"	262	1	0
46	Haute-Garonne	N 43° 20' 05.4"	E 0° 53' 37.6"	334	0	0
47	Ariège	N 43° 20' 2.13"	E 1° 20' 30.969"	250	0	0
48	Haute-Garonne	N 43° 20' 38.7"	E 0° 54' 11.6"	342	0	0
49	Haute-Garonne	N 43° 20' 51.743"	E 1° 37' 47.421"	286	1	1
50	Haute-Garonne	N 43° 21' 22.284"	E 1° 17' 44.379"	279	0	0
51	Haute-Garonne	N 43° 23' 39.8"	E 1° 03' 59.8"	335	0	0
52	Gers	N 43° 28' 22.1"	E 0° 49' 33.4"	301	0	0
53	Gers	N 43° 29' 52.3"	E 0° 50' 28.9"	275	0	0
54	Haute-Garonne	N 43° 30' 21.26"	E 1° 26' 45.187"	232	1	1
55	Aveyron	N 43° 53' 48.696"	E 2° 21' 0.036"	424	0	0
56	Tarn	N 43° 53' 48.7"	E 2° 21' 00.0"	421	0	0
57	Tarn	N 43° 56' 16.663"	E 2° 05' 3.069"	171	0	0
58	Tarn	N 43° 59' 36.022"	E 2° 05' 58.84"	300	1	1
59	Tarn	N 43° 59' 51.693"	E 2° 07' 18.874"	318	0	0
60	Tarn	N 44° 00' 24.156"	E 2° 05' 44.926"	315	0	0
61	Tarn	N 44° 00' 40.72"	E 2° 16' 29.964"	497	0	0
62	Tarn	N 44° 01' 11.92"	E 2° 11' 15.241"	350	1	1
63	Tarn	N 44° 01' 18.426"	E 2° 04' 19.966"	312	1	1
64	Tarn	N 44° 03' 1.767"	E 2° 17' 43.103"	527	1	1
65	Tarn	N 44° 03' 35.881"	E 2° 18' 19.369"	583	1	1
66	Tarn	N 44° 04' 25.0"	E 2° 19' 18.0"	581	1	0
67	Tarn	N 44° 03' 35.9"	E 2° 18' 20.9"	581	0	0
68	Tarn	N 44° 04' 14.9"	E 2° 20' 09.6"	592	1	0
69	Tarn	N 44° 04' 50.7"	E 2° 20' 31.9"	583	1	0
70	Aveyron	N 44° 06' 39.319"	E 2° 22' 24.164"	615	1	1
71	Aveyron	N 44° 14' 40.7"	E 2° 22' 02.1"	615	1	0

Overwintering sites are far from being safe
haven for ladybirds
(Coleoptera: Coccinellidae):
insights with *Hippodamia undecimnotata*
(Schneider)

In preparation



Photographie II. Site d'étude de Labastide-Gabausse. Les chutes de neige restent exceptionnelles dans cette région. L'hiver 2013-2014 est l'exception qui confirme la règle : les coccinelles ont passé près de 15 jours sous 20 centimètres de neige.

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Overwintering sites are far from being safe haven for *Hippodamia undecimnotata* (Schneider) (Coleoptera: Coccinellidae)

Running title: Susset et al.: Overwintering conditions for *Hippodamia undecimnotata*

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ABSTRACT

Aggregations during diapause are common among terrestrial arthropod species. They are usually considered as a defence strategy or a way to resist unfavourable abiotic conditions. However, systematic studies on the abiotic conditions as close as possible of arthropods in their aggregation sites (we will later use the word “microsite” for the accurate location of ladybirds, our biological model) and individuals’ survival in the aggregations are lacking. In this work, we studied whether aggregation microsites of the ladybird *Hippodamia undecimnotata* (Schneider) provide suitable abiotic conditions for its survival. We recorded hourly temperature and relative humidity throughout the overwintering aggregation period in the microsites at three overwintering aggregation sites and investigated if they 1) favour ladybird survival, and 2) prevent the proliferation of ectoparasitic fungi, a lethal threat for ladybirds. We found that overwintering microsites did not offer direct suitable conditions in terms of temperature for ladybird survival and/or presented relative humidity and temperature conditions favourable to ectoparasitic fungi. The adaptive value of aggregations is discussed.

Key words: abiotic conditions, microsite, diapause aggregations, ectoparasitic fungi, entomopathogens

INTRODUCTION

Aggregations during diapause are observed in several arthropods, including harvestmen, butterflies, and bugs (Holmberg *et al.* 1984; Denlinger 1986; Malcolm and Zalucki 1993). They are commonly thought to be a defence strategy (Vulinec 1990) or a way to resist unfavourable abiotic conditions (Holmberg *et al.* 1984; Pinheiro *et al.* 2008; Brower *et al.* 2008). By gathering, relatively immobile individuals can for instance benefit from dilution effects or confusion effects protecting them from predators (Vulinec 1990). Moreover, arthropods may either aggregate to create a favourable microclimate or converge to places that offer them favourable abiotic conditions (Holmberg *et al.* 1984; White *et al.* 1998a, b). For instance, the formation of aggregations in wet locations has been shown to promote water conservation and to avoid desiccation in the tropical fungus beetle *Stenotarsus rotundus* Arrow (Coleoptera: Endomychidae) and several other species (Yoder *et al.* 1992; Klok and Chown 1999; Broly *et al.* 2014). Another example is the monarch butterfly *Danaus plexippus* (L.) (Lepidoptera, Nymphalidae): it is found overwintering in areas with low precipitation and moderated diurnal temperatures, which are key environmental conditions for survival (Anderson and Brower 1996; Oberhauser and Peterson 2003). *D. plexippus* is even able to discriminate between suitable and non-suitable microsites once at the overwintering site (Brower *et al.* 2011).

In ladybirds (Coleoptera, Coccinellidae), an insect family comprising many species displaying aggregations while in diapause (Majerus 1994; Hodek 2012), aggregation site selection during either hibernation (i.e. diapause in winter; Hagen 1962, Tauber *et al.* 1986) or aestivation (i.e. diapause in summer, Hagen 1962) is still an enigma. Most of the ladybirds aggregate in the vicinity of their breeding sites (Hemptonne 1988) but some long-distance migrants also exist (Hodek *et al.* 1993; Hodek 2012). For the later the migration ending points are located at the top of hills or mountains, such as in the Rocky Mountains in the USA, in the Alps or in the Pyrenees in France (Hagen 1962; Iperti 1966). Furthermore, the aggregations of

some ladybird species comprise huge numbers of individuals, while they only count few individuals in other (Ceryngier 2015). Variability also appears at species level. The case of the well known charismatic *Coccinella septempunctata* L., a Palearctic species, is noteworthy: this species displays along its range populations with different diapause behaviours, forming large aggregations in mountainous areas in Southern Turkey that they reach after long-distance migration. In Western and Central Europe they remain in low lands, close to their breeding sites, in small groups hidden in the litter (Bodenheimer 1943; Hemptinne 1988; Ceryngier 2015; Güven *et al.* 2015). This high diversity of situations, both at species and population levels, casts some doubts on the usual ecological explanations for the evolution of diapause aggregations: do ladybirds' diapause aggregations provide suitable conditions for survival?

In overwintering aggregation sites, ladybird mortality has been seen to be high (El Hariri 1966; Labrie *et al.* 2008; Ceryngier 2000). Explanations for this mortality are reserve depletion due to flight migration and/or basic metabolism, disease, and predation (Chapman *et al.* 1955; Harrison and Roberts 2000; Nalepa and Weir 2007). Ectoparasitic fungi such as *Beauveria bassiana* (Balsamo) Vuillemin (Hyphomycetes: Deuteromycotina) or *Hesperomyces virescens* Thaxter (Ascomycetes: Laboulbeniales) are major causes of mortality killing up to 70-80% of the aggregated ladybirds (Nalepa and Weir 2007; Güven *et al.* 2015). Immunity, a costly physiological function, should also contribute to reserve depletion (Sheldon and Verhulst 1996; Siva-Jothy *et al.* 2005). Furthermore, we could expect additional mortality resulting from weather conditions. That is, the importance of the mortality factors in ladybirds' overwintering sites undermines the idea that they constitute an ideal refuge against detrimental weather conditions and natural enemies.

Hippodamia undecimnotata (Schneider) is an interesting ladybird model to address this question. It performs short as well as long-distance migrations and it hibernates in huge

groups at the base of prominent objects isolated at the summit of promontories, at low as well as high altitudes (Hagen 1962; Hodek *et al.* 1993; Hodek 2012). In France, *H. undecimnotata* experiences a long period of diapause: it hibernates from autumn until the following spring (Hagen 1962; Susset, pers. obs.). Once on top of promontories, it aggregates inside specific microsites such as crevices on rocks, vegetation, and dead trees or even phone poles (Hodek 2012). In this study, we investigate whether abiotic conditions (temperature and relative humidity) inside these overwintering aggregation microsites are 1) suitable for ladybird survival, and 2) detrimental for the proliferation of ectoparasitic fungi.

There are significant early works on the diapause habits of ladybirds, and above all the seminal work of Hodek and his colleagues (review in Hodek 2012 and Ceryngier 2015). However, to our knowledge, this is the first study to record abiotic conditions on an hourly base, throughout the diapause period, inside the microsites in several overwintering aggregation sites.

MATERIAL AND METHODS

Overwintering aggregation sites

The study took place during the 2014-2015 overwintering period at three sites where *H. undecimnotata* aggregates for more than 40 years: Marestaing “MAR” (Latitude: 43°34.90’N, Longitude: 0°59.99’E, Altitude: 202 m a.s.l.), Labastide-Gabause “LG” (Latitude: 44°2’N, Longitude: 2°6’E, Altitude: 260 m a.s.l.), and Mont Seigne “MS” (Latitude: 44°12’N, Longitude: 2°55’E, Altitude: 1128 m a.s.l.). Both MAR and LG sites are organic vineyards, surrounded by conventional crops; ladybirds aggregate in the crevices of vines. MS is a heathland dominated by *Calluna vulgaris* Hull, with low human disturbance during the overwintering period; ladybirds aggregate in the crevices of rocks. The 3 sites are in a sub-Atlantic climate zone, affected by both Mediterranean and mountain influences.

For each site, one microsite was studied. Ladybird abundance was recorded a week after the temperature and relative humidity started to be recorded. There were about 50, 550, and 2500 individuals at MAR, LG, MS, respectively. For 2 years, the number of ladybirds has been drastically decreasing in MAR for unidentified reasons. Before that, at least 450 ladybirds were recorded each year (Susset, pers. obs.).

Recording abiotic conditions

At each site, a HOBO[®] datalogger (Model U14-002, Onset Computer Corporation, Pocasset, Massachusetts) recorded temperature and relative humidity (RH) every 1h throughout the overwintering period.

The dataloggers were set up inside the microsite at the nearest of the ladybirds (less than 5 cm away) except at Mont Seigne. We feared that wild boars and voles, which are particularly abundant, might dislodge the data logger. We therefore installed it in a safer crevice 30 cm away from the ladybirds.

By comparing our field observations, data from the French national meteorological service with the data recorded by our data loggers, it appears that the simultaneous occurrence of temperature smaller than 3°C with a 100% RH indicates the presence of snow.

Thermal daily suitable conditions for the ladybirds and ectoparasitic fungi were also evaluated according to empirical data. No information on suitable abiotic conditions for hibernation was available for *H. undecimnotata*. However, information were available for *Harmonia axyridis*, a ladybird phylogenetically close (Magro *et al.* 2010), of similar body size and that shares the same breeding and overwintering habitats that *H. undecimnotata*. According to parameters found for *H. axyridis*, ladybird survival during diapause is better between -5°C and +5°C (Watanabe 2002; Hamedi and Moharramipour 2013). Ectoparasitic fungi require relative humidity above 80% and temperatures between 15°C and 35°C for spore germination,

mycelial growth, and sporulation while temperatures between -15°C and 10°C keep the spores viable for at least one year (Walstad *et al.* 1970; Lingg and Donaldson 1981).

Ladybird mortality

Dead ladybirds were collected from the sites and counted every month and when the last beetle had left the overwintering sites at LG and MS. These counts were summed up when the ladybirds had left their overwintering sites to estimate the cumulative mortality. Dead ladybirds were counted after the last ladybird had dispersed at MAR. The insect bodies were visually examined for the presence of fungal thalli.

Statistical analyses

We compared the effect of Date and Microsites on the daily temperatures with a linear model (LM), after transforming daily temperatures in degrees Fahrenheit (to avoid negative values, for statistical reasons). We analyzed the effect of Date, Microsite, and their interaction on the mean daily relative humidity with a GLM using a binomial error distribution. Then, we used a linear model with a binomial error distribution to compare the number of suitable days for ladybird survival between the 3 microsites. The temperature values between -5 and $+5^{\circ}\text{C}$ (i.e. between 23 and 41°F) were considered favorable for ladybird survival (value = 1) while those outside this range were unfavorable (value = 0). Thirdly, the effect of Date, Microsite, and their interaction on daily temperatures and daily relative humidity amplitudes were compared between the 3 microsites by GLMs with a Gamma error distribution. Finally, we used a Chi-square test to investigate if there is a significant difference in the number of hours favorable to fungi sporulation between the overwintering microsites. Another Chi-square test was used to investigate the homogeneity of mortality proportions between the sites. We tested the correlation between ladybird cumulative relative mortality and the number of unfavorable

days in terms of temperature for ladybird survival at LG and MS sites along the overwintering period using a Pearson's correlation test.

All analyses were conducted in the R version [2.14] environment (R Development Core Team 2012).

RESULTS

During the overwintering period, the temperatures ranged between -2.9, -7, and -6°C (lowest values) and 19.6, 32.6, 34°C (highest values) for the three microsites at MS, LG, and MAR, respectively. The 3 microsites significantly differ in their mean daily temperatures and mean daily RH. Moreover, the mean daily temperatures and RH are significantly affected by the Date (Figs S1, S2; Table 1). The mean daily RH was significantly influenced by the Date × Microsite interaction while the mean daily temperatures were not (Table 1).

Table 1 Effects of the dates, the microsites, and their interaction on the mean daily temperatures and mean relative humidity.

	Df	F value	P
<i>Temperature</i>			
Date	1, 205	6.13	< 0.05
Microsite	2, 203	22.02	< 0.001
Date × Microsite	2, 201	1.07	0.25
<i>Relative humidity</i>			
Date	1, 205	72.96	< 0.001
Microsite	2, 203	311.48	< 0.001
Date × Microsite	2, 201	15.75	< 0.001

The number of days with daily suitable temperatures favourable for ladybird survival is significantly different between the 3 overwintering microsites ($F_{2,527} = 38.76$, $P < 0.001$) (Table 2; Fig. S1). The microsite at MS has the highest number of suitable temperature days, followed by LG and then MARS (Table 2). Temperatures under - 5°C were recorded only once at LG and MAR (Fig. S1).

Table 2 Numbers of days and hours with suitable temperature for the ladybird survival according to the [-5°C; +5°C] interval recorded during the overwintering period at the 3 overwintering microsites.

Location	Recording dates	Recording days	Days with suitable temperatures for ladybirds (Proportion)	Recording hours	Hours with suitable temperatures for ladybirds (Proportion)
LG	Nov. 2014, 17 th - May 2015, 4 th	169	55 (32.5%)	4035	1422 (35%)
MS	Nov. 2014, 11 th - May 2015, 5 th	176	113 (64%)	4186	2723 (65%)
MAR	Nov. 2014, 7 th - May 2015, 10 th	185	38 (20.5%)	4441	1081 (24%)

The daily temperature amplitudes through the overwintering period vary with Date and Microsite but there is no Date \times Microsite interaction. Similarly, the daily RH amplitude is influenced by Date, Microsite, but not by the Date \times Microsite interaction (Table 3).

MS experiences the lowest temperature and RH amplitudes during the overwintering period (Fig. 1). The widest temperature amplitudes were recorded during the entire period at MAR while the widest temperature amplitudes occur mainly in the second part of the overwintering period at LG.

MS is the only site where snowfalls occurred during the studied overwintering period. The overwintering ladybirds spent there 50 days under the snow cover.

Table 3 Effects of the Date, Microsite, and their interaction on the mean daily temperature amplitude and mean daily relative humidity amplitude

	Df	F value	P
<i>Temperature amplitude</i>			
Date	1,522	11.03	< 0.001
Microsite	1,520	132.32	< 0.001
Date \times Microsite	2,518	0.75	0.47
<i>Relative humidity amplitude</i>			
Date	1,523	21.88	< 0.001
Microsite	2,521	57.60	< 0.001
Date \times Microsite	2,519	2.474	0.08

During the overwintering period, 34, 132, and 146 hours, recorded over 9, 25, and 21 days, were favourable to the sporulation of the ectoparasite fungi (*B. bassiana*) for MS, LG and MAR, respectively. The number of favourable hours for fungus sporulation is

significantly different between the 3 microsites ($\chi^2 = 70.99$, $df = 2$, $P < 0.001$), with MS providing the fewer hours favourable to fungus sporulation.

There were 457 (out of 2507; 18%), 152 (out of 552; 28%), and 12 (out of 51; 24%) dead ladybirds recorded at MS, LG, and MAR, respectively. 12%, 84%, and 16% of dead ladybirds were affected by ectoparasite fungi. The ladybird mortality is significantly different between the 3 overwintering microsites ($\chi^2 = 24.93$, $df = 2$, $P < 0.001$), with ladybird mortality being the highest at LG and MAR. We found a positive and highly significant correlation between the ladybird cumulative mortality and the number of unfavourable days at both sites (for LG: Pearson's correlation test, $r = 0.90$, $P < 0.001$; for MS: Pearson's correlation test, $r = 0.94$, $P < 0.001$; Fig. 2).

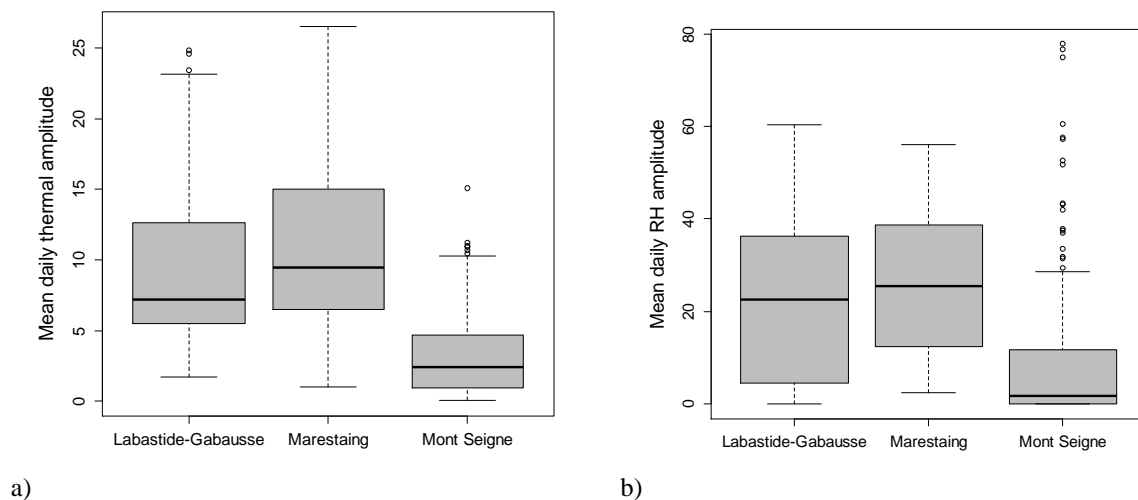


Fig. 1 Daily amplitude minimal, maximal, and mean of a) temperature (°C) and b) relative humidity (%) calculated at the 3 overwintering microsites of *H. undecimnotata*

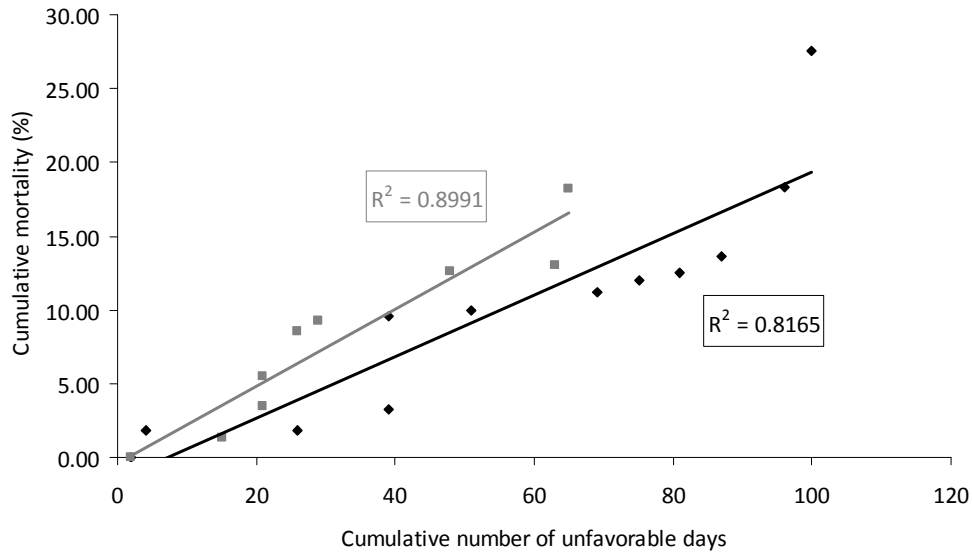


Fig. 2 Relationship between ladybird cumulative relative mortality and the number of unfavourable days recorded at each overwintering site. Black dots: LG data; Grey dots: MS data.

DISCUSSION

We investigated whether *H. undecimnotata* overwintering aggregations are located in sites providing them with favourable abiotic conditions for survival by recording the temperature and the relative humidity as close as possible to the ladybirds, in the microsites where they aggregate in three overwintering sites. This allows us to characterize the abiotic conditions experienced by the ladybirds and their within-day amplitudes at a fine-scale. Our results showed that there are significant differences between microsites in terms of temperature and RH suitable conditions from ladybirds' point of view. Moreover, temperatures and relative humidity recorded at all the three studied sites are unfavourable for ladybird survival for a prolonged period, representing 36 to 80% of the time spent by the ladybirds at their overwintering sites.

Mortality rates were high in our study but lower than those found in previous studies (El Hariri 1966; Barron and Wilson 1998; Labrie *et al.* 2008; Güven *et al.* 2015). Although our work is preliminary and should consider a higher number of sites, we found a positive

correlation between ladybird mortality and the number of unfavourable days experienced by ladybirds. According to our results, overwintering microsites did not offer suitable temperatures for ladybird survival. In addition, abiotic conditions are largely favourable to ectoparasitic fungi parasitizing the ladybirds.

Contrary to previous hypotheses (e.g. Honěk *et al.* 2007; Durieux *et al.* 2014), mild temperatures do not benefit overwintering ladybirds. Low temperatures are probably safer (Hagen 1962; Sinclair 2015). If stable, they decrease energy consumption, lower the mortality rate, and as a consequence increase spring fecundity (Irwin and Lee Jr 2003; Sinclair 2015). What affects insect survival is temperature variability and the occurrence of mild-temperature spells (Colinet *et al.* 2015; Marshall and Sinclair 2015), as they drive insects to never fully suppress their metabolism and spend more energetic resources (Hahn and Denlinger 2011; Sinclair 2015). The temperature was mainly variable at LG and MAR, and to a lower extent at MS probably because this last site is the only location where the ladybirds were insulated by a thick snow cap, which is an excellent insulator providing stable conditions (Geiger 1966 in Irwin and Lee Jr 2003; Zhang 2005). The first factor of high mortality could be the abiotic condition variation.

Concerning fungi proliferation, the RH was high in the three overwintering sites (79-95% on average) and temperatures high mostly at the beginning and at the end of the overwintering period. Ectoparasitic fungi are favoured by the simultaneous occurrence of warm temperatures (15-35°C) and high RH (90-100%) (Walstad *et al.* 1970). The abiotic conditions at the microsites thus provide suitable conditions for the development and propagation of ectoparasitic fungi. Ladybirds parasitized by ectoparasitic fungi were observed in the three studied sites with the highest rate observed at LG: it was up to 84% of prevalence among dead ladybirds and corresponds to rates recorded by Güven *et al.* (2015) in *C. septempunctata*. The infection by lethal ectoparasitic fungi is the second risk faced by

ladybirds at their overwintering sites as shown here for *H. undecimnotata* and in other studies for *Adalia bipunctata* (L.), *Harmonia axyridis* (Pallas) and *C. septempunctata* (Mills 1981; Nalepa and Weir, 2007; Güven *et al.* 2015).

In our study, MS which is the site at the highest altitude offers the best abiotic conditions compared with LG and MAR: the temperatures are colder and the inter- and intraday temperature amplitudes are the narrowest. We would thus expect *H. undecimnotata* to preferentially overwinter in high-altitude sites. However and contrary to our expectations, not all the ladybirds overwinter in sites at high altitudes. Indeed, we identified a total of 22 overwintering aggregation sites in South-western France (Susset, personal observation): 68% of them range at altitudes between 190 m and 350 m, meaning that most of the sites have the same sub-suitable fluctuating abiotic conditions like in LG and MAR.

According to our preliminary findings, the overwintering sites of *H. undecimnotata* are not of the highest quality in terms of the abiotic conditions, but this should be confirmed by more extensive studies. Overwintering sites might therefore not be safe haven. The reasons why ladybirds gather in these sites remain to be addressed. A first idea could be that *H. undecimnotata* that prefers overwintering at the top of promontories (Hagen, 1962; Hodek 1973; Hodek, 2012; Ceryngier, 2015) is constrained by its dispersal capacity and could have to stop at a promontory site close to its breeding site. Nevertheless, *H. undecimnotata* displays long-distance migration (Hodek *et al.* 1993) and it seems likely that it can visit several overwintering sites before aggregating but this remains to be addressed. Another reason could be that ladybirds are not limited by their dispersal capacity but seek sites for mating purpose, as suggested by Taylor (1984) and Majerus (1994) or for other resources, that remain to be identified. Although the sites where they spent the overwintering period are bad for them, ladybirds keep aggregating there because they may find their resources or mates in these

places. These resources or mates might be at the origin of overwintering aggregations in *H. undecimnotata* and ladybirds' diapause aggregations in general.

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SUPPORTING INFORMATION

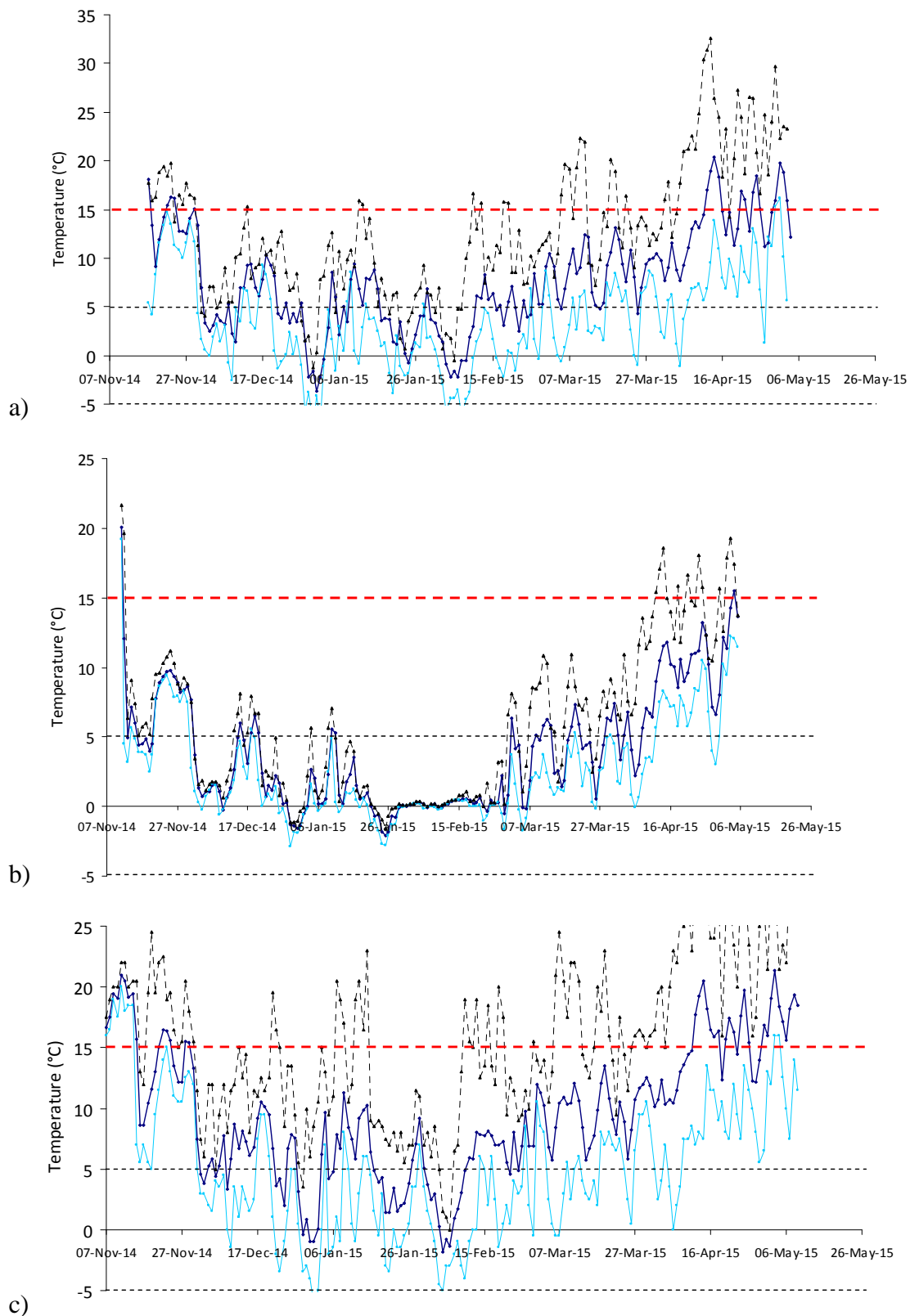


Fig. S1 Daily minimal, mean, and maximal temperatures at Labastide-Gabausse (a), Mont Seigne (b), and Marestaing (c). Dark blue full line: mean daily temperatures, black dotted line: max daily temperatures; blue full line: minimum daily temperatures. Interval between the dotted lines represents the optimal range of temperatures for ladybirds. The red dotted lines indicate the lower threshold of the optimal range of temperature for ectoparasite sporulation

SUPPORTING INFORMATION

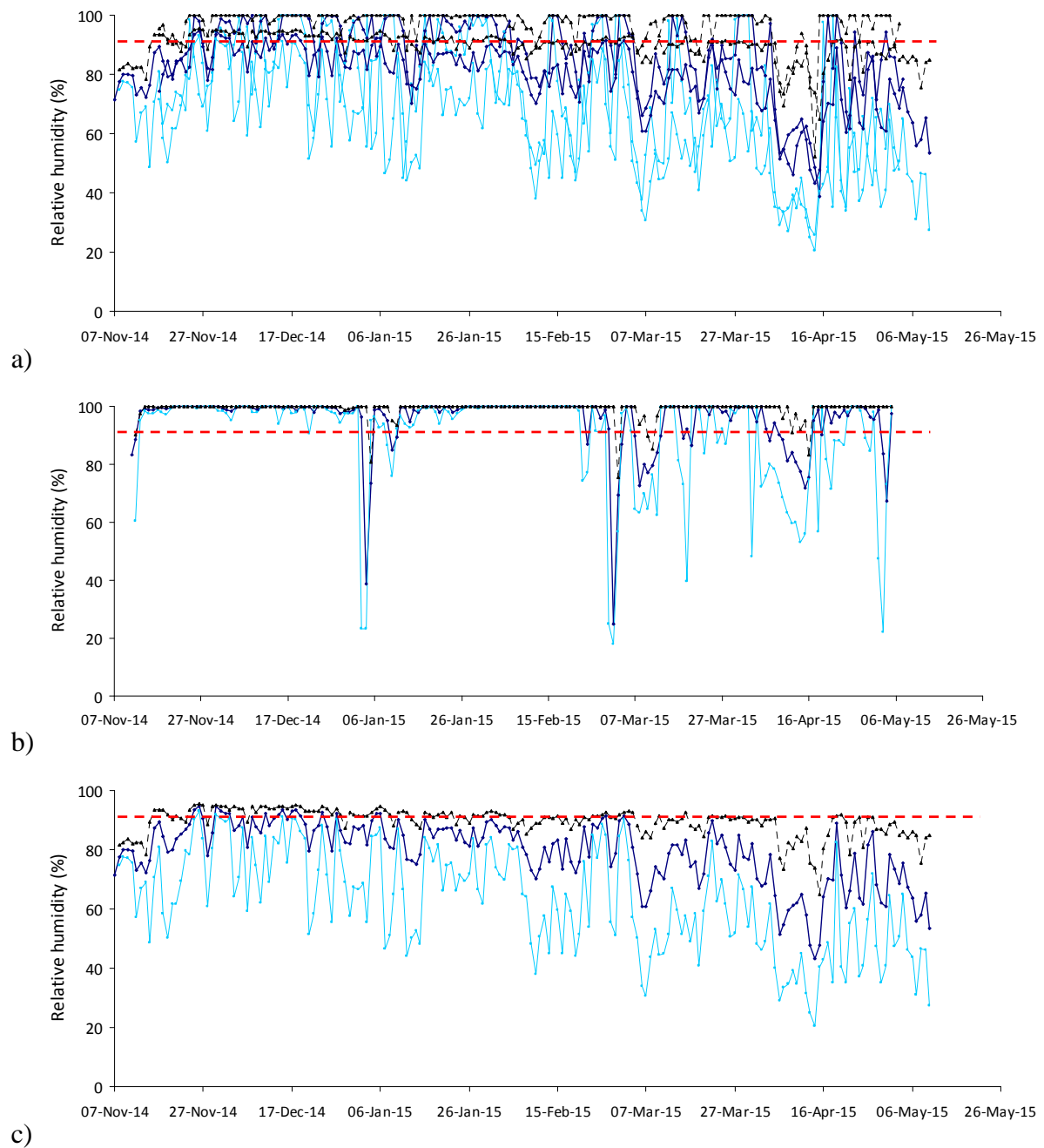


Fig. S2 Minimal, mean, and maximal daily relative humidity at Labastide-Gabausse (a), Mont Seigne (b), and Marestaing (c). Dark blue full line: mean daily RH; black dotted line: max daily RH; blue full line: minimum daily RH. The red dotted lines indicate the lower threshold of the optimal range of relative humidity for ectoparasite sporulation.

Is the evolution of insect overwintering aggregations sexually driven?

In preparation



Photographie III. Accouplement(s ?) de coccinelles dans une agrégation de diapause (Marestaing, mars 2013)

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ABSTRACT

Aggregation during diapause is a common phenomenon in arthropods that remains poorly understood. To date, aggregations have mainly been studied under the traditional economic approach (i.e. aggregation initially evolved because individuals directly benefited from aggregating). The most commonly claimed benefit is that survival probability is higher in aggregations. In this study, we investigated whether sexual selection could drive the evolution of aggregations in arthropods. We studied the overwintering aggregations of the ladybird *Hippodamia undecimnotata* (Schneider, 1792), an aphidophagous species from Southern and Eastern Europe as well as Asia. We collected ladybirds at 3 aggregation sites in Southwest France, during two overwintering periods (2013-2014 and 2014-2015). We checked their reproductive status through the counting of viable sperm cells in the sperm storage organs of both males and females. We also investigated the mating behaviour at the aggregation sites. We found that males have a high quantity of numerous viable sperm cells (70-95%) in their testes throughout the overwintering periods. In contrast, most females had empty spermatheca at the onset of the aggregations but left with numerous viable sperm in their spermatheca in early spring. Frequent mating behaviour was also observed towards the end of the overwintering period, few weeks before dispersal. These results lead us to suggest that overwintering aggregations are also part of the mating system of this species and could thus be viewed as actual leks. We thus discuss the links with the hidden lek and the commodity selection hypotheses initially proposed to explain breeding aggregations of vertebrates. This would suggest that the seeking of sexual mates could have been involved in the evolution of overwintering aggregations.

KEY-WORDS

Overwintering aggregations, mating, reproductive strategy, ladybird, diapause, hidden lek

INTRODUCTION

Group living is a common feature in the animal kingdom (Krause & Ruxton 2002; Parrish & Edelstein-Keshet 1999). Examples include insect swarms, fish school, ungulate herds, colonial breeding in birds and sessile organisms, and many others (Parrish & Hamner 1997). Once aggregated, individuals benefit from protection against predators, better food acquisition, or mate choice, among other identified potential benefits (Krause & Ruxton 2002; Danchin et al. 2008). Animal aggregations are often temporary and seasonal. They sometimes constitute responses to unfavourable long-term environmental conditions. In Arthropod, seasonal aggregations generally consist of monospecific clusters of sometimes high numbers of individuals for several months, and are located in the same places year after year (e.g. Holmberg et al. 1984; Malcolm & Zalucki 1993; Roubik & Skelley 2001; Toyama et al. 2006; Dzerefos et al. 2009; Hodek 2012). One of the most famous cases of arthropod seasonal aggregations is the huge meetings of the monarch butterfly *Danaus plexippus* (L.) (Lepidoptera: Nymphalidae) in the forests of Mexico and Florida (Malcolm & Zalucki 1993). Nevertheless, although seasonal aggregations are a fascinating phenomenon ultimate mechanisms for their formation are still poorly understood (Kitching & Zalucki 1981; Wells et al. 1990; Sabu et al. 2008).

A life-history trait that is often linked to arthropod seasonal aggregation is diapause, which is considered an arthropod's chief strategy to escape unfavourable seasons (Danks 1987; Bradshaw 1990). Gregarious behaviour during harsh periods is traditionally viewed as an additional adaptive strategy to increase survival. A general assumption is that gathering in the course of diapause increases the protection of the relatively immobile individuals against predators (Monteith 1982; Holmberg et al. 1984) owing, for instance, to increased intensity of aposematic signals (Pasteels et al. 1983; Beatty et al. 2005) or dilution effects (Vulinec 1990). Nevertheless, these benefits are far from clear-cut. For instance, in the monarch butterfly *D. plexippus* per capita predatory attack rates are higher for individuals in group than in isolation (Brower et al. 1977) with only those in the centre of the aggregations really benefiting from protection against predators (Brower et al. 1985). In the firebug *Pyrrhocoris apterus* L. (Hemiptera: Pyrrhocoridae), aggregated individuals are better protected than solitary ones but the predation rate varies according to geographical regions (Svádová et al. 2014; Exnerová et al. 2015), undermining the idea that aggregations have an exclusive anti-predatory function. Finally, disease transmission is ubiquitous in groups (Krause & Ruxton 2002) and pathogens or predators are common at the aggregation sites (Leong et al. 1992; Alonso-Mejía et al. 1998; Glendinning et al. 1988).

Aggregations during diapause may also generate favourable microclimates (Wells & Wells 1992; Brower et al. 2008). Aggregations were shown to promote water conservation (Tanaka et al. 1988; Yoder et al. 1992; Klok & Chown 1999) or lead to increase group temperature (Klok & Chown 1999). However, the temperature and humidity effects in many seasonal aggregations are tiny: in the monarch, they represent less than 1°C and 1% of relative humidity (Brower et al. 2008). Moreover, although cold is often viewed as detrimental to survival, diapausing arthropods have the physiological capacity to support low temperatures (Pullin 1996; Berkvens et al. 2010; Rozsypal et al. 2013). In fact, they seem more affected by the alternation between low- and warm-temperature events than by the intensity and duration of cold spells (Marshall & Sinclair 2015). All these observations cast some doubts on the general belief that diapause aggregations provide a net benefit in terms of thermoregulation.

To date, no study has established unambiguously the evolutionary mechanisms of arthropods aggregations (Wells & Wells 1992). The most common approach of these aggregations has focused on survival. However, there are other major determinants of fitness underlying the formation of aggregations that have been overlooked in explaining the evolution of aggregation (Danchin & Wagner 1997; Doligez et al. 2003; Danchin et al. 2008). For instance, the hidden lek hypothesis states that animal aggregations can be driven by sexual selection and result from the settlement of promiscuous males on sites where they are most likely to encounter female mates (Bradbury et al. 1986; Wagner 1993, 1998). According to that hypothesis, by joining one particular site, males and females are not looking for aggregation *per se* but for high quality individuals of the opposite sex (Danchin et al. 2008). Alternatively, aggregations may be the mere by-product of every individual seeking similar habitat (the “Habitat copying hypothesis”, Danchin et al. 1998). More generally, aggregations may result from all individuals seeking the same commodities to grow, survive, and breed (the “Commodity selection hypothesis”, Danchin & Wagner 1997).

Although these last hypotheses were developed in vertebrates, they can be transposed to insects. In particular, arthropod aggregations during diapause may be a by-product of individuals converging to specific meeting points to both find the necessary commodities to both survive the winter and find and choose mates (e.g. Isvaran & Ponkshe 2013). This view does not exclude that arthropods’ aggregations now convey other benefits. It only posits that these classically studied benefits are not at the very origin of aggregation formation, but rather, are mere by-products of aggregation once they are formed as a consequence of every

individual selecting the main commodities for reproduction (Danchin & Wagner 1997). Once these aggregations formed, these by-product benefits can participate to their maintenance.

In this context, ladybirds (Coleoptera: Coccinellidae) are interesting biological models to study the evolutionary origin of aggregations. Worldwide, many ladybird species aggregate in response to seasonality (Hodek 2012), either hibernating and/or aestivating, but few evolutionary explanations have been put forward. According to Majerus (1994), ladybirds aggregate either to regulate their microclimate, or to accentuate the aposematic signal sent to the predators, thus increasing survival. Interestingly, Taylor (1984) and Majerus (1994) also hypothesized that ladybirds benefit from the proximity of individuals of the opposite sex to mate before dispersing, but this was never tested. These authors plus Hagen (1962) noted that copulations often occur in overwintering aggregations in several ladybird species. Moreover, several authors found that females in aggregations had no sperm at the spermatheca in the beginning of diapause and that the proportion of mated females (i.e. with sperm in the spermatheca) gradually increased over the course of the overwintering period (Hodek & Landa 1971; Hemptinne & Naisse 1987; Ceryngier et al. 2004).

Here, we tested the hypothesis that aggregations of ladybirds can be sex-driven. In other words, our hypothesis was that ladybirds aggregate during summer or winter diapauses to mate before resuming activity, at the end of the harsh season and before dispersing towards breeding sites. We studied the overwintering aggregation behaviour of *Hippodamia undecimnotata* (Schneider 1792), an aphidophagous ladybird species from Southern and Eastern Europe and Asia (Iablokoff-Khnzorian 1982; Hodek 2012).

We investigated whether mating occurs at the aggregation sites and, if so, in which proportions and when. To do so, we counted viable sperm cells in male gonads and female spermatheca (their sperm storage organ) and *bursa copulatrix* (sperm deposition organ) over the whole overwintering period using sperm count method (Perry & Rowe 2010) coupled with a coloration method enabling to distinguish live from dead sperm cells (Damiens et al. 2002). We also investigated the ovarian status of sampled females to evaluate their maturity level over time. Five clusters from 3 sites were studied over two years. For one additional cluster, the reproductive status of the entire dispersing female population was also evaluated. At all sites, the study was backed up by regular direct observations of mating behaviour. If aggregation sites are mating places, we expected high proportions of males to have numerous viable sperm cells in their gonads and females to arrive at the aggregation sites without sperm cells in the spermatheca and the *bursa copulatrix* but leave the sites in a mated state. Finally,

we predicted high frequencies of mating events with higher frequencies towards the end of the overwintering period.

MATERIAL & METHODS

Studied sites

The study took place over two consecutive overwintering periods from November 2013 to April 2014 (Year 1) and from November 2014 to April 2015 (Year 2) at three sites in South-west France: Labastide-Gabause (LG, Latitude: 44°2'N, Longitude: 2°6'E, Altitude: 260 m a.s.l.), Saint-Michel-de-Lanès (SML, Latitude: 43°19'N, Longitude: 1°45'E, Altitude: 315 m a.s.l.), and Mont Seigne (MS, Latitude: 44°12'N, Longitude: 2°55'E, Altitude: 1128 m a.s.l.). LG is an organic vineyard and ladybirds aggregate in cracks on the vine stocks present at the highest points. SML is situated on a conventional crops area and ladybirds aggregate at the base of a phone pole next to a road. MS is a heathland dominated by *Calluna vulgaris* Hull (Ericaceae), with low human disturbance during the winter and ladybirds aggregate in the crevices of the masonry of two orientation tables.

Field ladybird sampling

We sampled ladybirds in the 3 aggregation sites, from the beginning to the end of the overwintering period. Samples were taken in November, January, March, and April in both years 1 and 2. For each aggregation site, samples were collected in two clusters at least 10 m apart from each other, except at SML where only 1 cluster was available.

We carefully collected the ladybirds with fine tweezers to avoid disturbing the remaining ladybirds. After sampling, we brought them to the laboratory in a portable fridge at 10°C and they were stored in a climate-controlled room at 10°C, Light:Darkness ratio 0:24 for 24h, until required for dissections.

297 males and 340 females were analysed in Year 1; 408 males and 438 females were analysed in Year 2.

Dissection of ladybirds and sperm collection

We sexed ladybirds under a stereo-microscope, following Hodek et al. (2012). We carried out dissections the day following the sampling, in a cavity slide. The ventral tegument was teased apart with fine tweezers and 100 µl of PBS were added to avoid cytolysis and organ retraction. In males, we removed the whole reproductive apparatus, including the testes and the seminal vesicles; in females, we removed the spermatheca and the *bursa copulatrix*. We

ruptured the reproductive organs to allow sperm release. As the spermatheca is a chitinous structure, we pressed it with forceps to force the ejection of sperm. Then, we pipetted 100 μ l of PBS containing the sperm from the slide into a microcentrifuge tube. We washed the cavity slide with PBS (400 μ l for males; 100 μ l for females) that was added into the microcentrifuge tube. The tube was then vortexed at 600tr/min for 30 min. This allows the remaining sperm cells to leave the reproductive organs without increasing sperm mortality (Gress & Kelly 2011).

Sperm cell number

We counted sperm cells under a dark field phase contrast microscope at 400x magnification. We counted the number of sperm cells in two samples for each individual; count repeatability was high (Pearson's correlation test: $r = 0.82$; $P < 0.0001$). The number of sperm cells detected in both samples was thus summed and multiplied by the dilution factor to estimate the total number of sperm cells. Twenty to twenty-five males and females were analyzed per sampling event for each site.

Sperm cell viability

The live/dead sperm cells viability assay was performed using Molecular Probes reagent, adapted from Damiens et al. (2002) and García-González & Simmons (2005). Ten μ l of sperm were pipetted and mixed with an equal volume of 1:50 diluted 1 mM SYBR-14 and were left in the dark for 10 min before 2 μ l of 2.4 mM propidium iodide was added. The sample was then incubated in the dark for 10 minutes and then observed under a fluorescence microscope (blue excitation filter at $\lambda = 490$ nm). Live sperm cells are green stained with SYBR-14, a membrane permanent nucleic acid stain, while dead ones, with damaged membranes, are stained red with propidium iodide (Damiens et al. 2002). To assess the repeatability of the measurement, sperm cells viability (the proportion of viable: non viable sperm cells) was assessed in two samples for each of 21 ladybirds, and was found to be high (Pearson's correlation test: $r = 0.79$; $P < 0.0001$).

Ovarian status

We investigated the ovarian status for each female for which we had sperm cell numbers and viability. Ovarian development was assessed according to a 5-degree scale described by Okuda & Hodek (1989):

- Stage 1: inactive ovary with ovarioles containing no oocytes,

- Stage 2: ovarioles with the first, transparent oocytes,
- Stage 3: ovarioles with small, vitellinized oocytes,
- Stage 4: ovarioles with vitellinized oocytes,
- Stage 5: ovarioles with matures oocytes.

Females with ovarian status is under stage 2 were considered in diapause.

Exhaustive sampling of females at the time of dispersal

To investigate the proportion of mated females when leaving the aggregation site, we caught all dispersing ladybirds in a third cluster at the LG site, not used for the other parts of the study, using an interception entomological trap. This trap, inspired by Sarthou (2009), is an asymmetric tent without a base. The trap was set up fitting tightly to the ground. Insects emerging from the area below the trap were collected at the top of the trap, in a bottle. The trap was set up when the ladybirds were still immobile and densely packed in the aggregation (in both year in March), and removed once all the ladybirds had dispersed (in both year in May). During this period, the collecting bottle was checked 3 times a week and emptied when at least one ladybird was trapped. Ladybirds were brought back to the laboratory and their spermatheca and *bursa copulatrix* content analysed the following day, as described above.

Ladybird behaviour at the LG aggregation site

We observed ladybird behaviour at each sampling date and once a week on a sunny day, from March to the beginning of May 2014 and 2015, at Labastide-Gabause between 9:00 am and 6:00 pm. We performed control visits once a month during raining days to check whether mating occurred only during sunny days. In 2014, we followed two clusters of about 200 and 70 ladybirds each and recorded movements across the aggregation and mating events. In 2015, we followed one cluster of about 155 ladybirds. Mating ladybirds were marked with white paint on the elytra.

Low Developmental Threshold (LDT) for *H. undecimontata* is 11°C (Honek & Kocourek 1988). A HOBO[®] datalogger (Model U14-002, Onset Computer Corporation, Pocasset, Massachusetts) was set up near the cluster used for the exhaustive sampling. Temperature and relative humidity were recorded every hour daily from January to May, 2014 and 2015. The number of accumulated day-degrees (ADD) until first mating was then calculated as followed:

$$ADD = (T_{\max} + T_{\min})/2 - LDT$$

where T_{\max} is the highest temperature recorded and T_{\min} is the lowest temperature recorded in a given day (Pruess 1983).

Statistical analyses

We analysed the Site, Period, Year, and Cluster effects, and their interaction on the proportion of males and females having viable sperm cells in their reproductive organs, as well as on sperm cell number and viability with GLMMs (Generalized Linear Mixed Models). We assumed a binomial error distribution for the proportion of individuals with sperm cells and for the sperm viability (Zuur et al. 2009). We used a penalized quasi-likelihood GLMM (glmmPQL) with quasi-Poisson error distribution for the sperm cell number. In addition, we investigated the effects of Site, Year, and their interaction on the sperm cell number and viability in the females sampled the last sampling date (i.e. April) using a generalized linear model (GLM) with a quasipoisson and a binomial error distribution, respectively to compare the number and viability of gametes across sites and years. We investigated the effects of Site, Period, Year, and Cluster on female ovary maturation with a Generalized Linear Mixed Model (GLMM) assuming a Poisson error distribution using the lme4 package.

In all models, Site, Period, and Year were entered as fixed effects while Cluster was considered as a random factor. Period was considered as a 4 levels ordered variable: November, January, March and April. Due to important rainfalls in March 2014 at SML, the aggregation site was destroyed. So, the site effect had only 2 levels in year 1: LG and MS and 3 levels in year 2: LG, MS, and SML.

We included all main effects and interaction terms in all the initial models, which were then simplified by backward selection. Only significant terms are reported in the Results section.

All analyses were conducted in the R version [2.14] environment (R Development Core Team 2012), using the lme4 (Bates et al. 2008) and MASS (Ripley et al. 2015) packages.

RESULTS

Numbers of viable sperm cells in males

When analysing the number of males with sperm cells in their testes, the three-way interaction Period \times Site \times Year was non-significant (GLMM: $\chi^2_2 = 0.000$, $P = 1$). The number of males with sperm cells in their testes varied in relation to the 2 two-way interactions involving Period (Period \times Year: $\chi^2_3 = 6.75$, $P < 0.001$; Period \times Site: $\chi^2_5 = 2.70$, $P = 0.018$). In Year 1, the highest proportion of males with sperm cells in their testes was found from January to

April in one aggregation (MS) but in March in the other (LG). By contrast, this proportion was the highest from the beginning of the overwintering period until March in all the sites in Year 2 (Table 1).

The three-way interaction Period \times Site \times Year had a significant effect on the number of sperm cells in males (quasi-Poisson GLMM: $\chi^2_3 = 7.87$, $P < 0.001$). In Years 1 and 2, sperm cell number varied according to the Period \times Site interaction but the tendency was weaker in Year 2 (quasi-Poisson GLMM: $\chi^2_2 = 2.51$, $P = 0.02$) than in Year 1 (quasi-Poisson GLMM: $\chi^2_5 = 8.66$, $P < 0.001$). Sperm cell number was the highest during the second half of the overwintering period in Year 1 but in Year 2 it was high both at the beginning of the first half of the overwintering period, and until March in SML (Table 1).

Finally, when analysing factors related to the viability of sperm cells in males, the three-way interaction Period \times Site \times Year was significant (GLMM: $\chi^2_2 = 3.43$, $P = 0.03$). In Year 1, the Period \times Site interaction was significantly related to the viability of sperm cells in males (GLMM: $\chi^2_2 = 2.43$, $P = 0.08$): sperm cell viability was the highest in January in LG while it was the highest in November in MS (Table 1). In Year 2, the Period \times Site interaction was also related to the viability of sperm cells in males (GLMM: $\chi^2_5 = 3.07$, $P = 0.009$): sperm cell viability increased over time in the 3 sites, but males in SML first underwent a decrease in their sperm cell viability in January before it increased in the following months (Table 1).

Female sperm cell number and viability

First, the interaction between Period and Year was significantly related to the proportion of females with sperm in the spermatheca and the *bursa copulatrix* (GLMM: $\chi^2_3 = 3.94$, $P < 0.01$). The other interactions were non-significant (three-way interaction: $P = 0.13$; two-way interaction Site \times Year $P = 0.26$; two-way interaction Period \times Site $P = 0.22$). Thus although the proportion of females having sperm cells in their spermatheca and *bursa copulatrix* increased over the winter (GLMM: $\chi^2_3 = 208.82$, $P < 0.001$; Figure 2), that trend differed between years. However, the general trend was clear: few females were mated at their arrival at the aggregation sites (Figure 1). This persisted until March, and then in April, most females were mated (Figure 1, Table 2). The exhaustive collection of departing females at LG showed that 91% and 83% out of 124 and 47 females in April 2014 and April 2015 respectively had sperm in their spermatheca and the *bursa copulatrix* (Figure 1). Furthermore, the proportion of mated females significantly differed among sites (GLMM: $\chi^2_2 = 15.54$; $P < 0.001$; Figure 1).

When studying the total sperm cell numbers in the spermatheca and the *bursa copulatrix* in April we found a significant Site \times Year interaction (quasi-Poisson GLM: $F_{1,142} = 8.56$; $P < 0.01$). The viability of sperm cells in April was also related by the interaction between Site and Year (GLM: $F_{1,139} = 26.08$; $P < 0.001$). Nevertheless, sperm cell viability in the spermatheca and *bursa copulatrix* was high ($> 80\%$) at the time of dispersal in both years (Table 2).

Ovarian status

The three-way interaction Period \times Site \times Year on the ovarian status was non-significant (GLMM: $\chi^2_3 = 1.78$; $P = 0.14$) but the interactions Period \times Site (GLMM: $\chi^2_6 = 4.96$; $P < 0.001$) and Period \times Year (GLMM: $\chi^2_3 = 42.33$; $P < 0.001$) were both significant.

In Year 1, the interaction Period \times Site was significant on the ovarian status (GLMM: $\chi^2_3 = 6.01$; $P < 0.001$). Females had immature ovaries (below stage 3) from their arrival at the aggregation site until January 2014 in all the 3 locations. A higher proportion of females had mature ovaries (stages 1 and 2) in March. In contrast, all dissected females in April 2014 had mature ovaries (stage 3 and beyond) (Figure 2). In Year 2, only the Period effect was significant on ovarian status (GLMM: $\chi^2_3 = 20.92$; $P < 0.001$). Some females had mature ovaries (at stage 3) in November. Then, females remained with immature ovaries all over the winter and spring before leaving the aggregation (Figure 3).

Ladybird Behaviour at the LG aggregation site

In both 2014 and 2015, the first walking movements were observed at the beginning of February. Before that, ladybirds remained immobile and densely packed inside the crevices. The first copulations were observed in March.

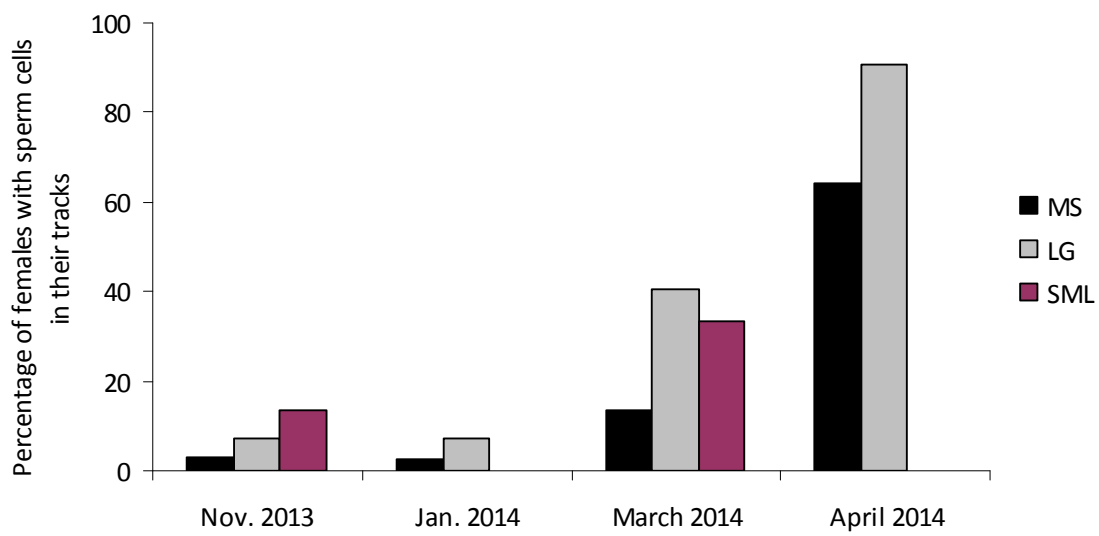
In 2014, over 16 non-consecutive days (representing a total of 144 hours of observation), a total of 46 and 21 different couples out of more than 219 and 208 individuals were recorded in cluster 1 and cluster 2, respectively. No mating were observed during raining days (over 40 hours of observation). Mating started when ladybirds accumulated a minimum of 14 accumulated day-degrees (Figure 4).

In 2015, over 14 non-consecutive days (representing 126 hours of observations), a total of 25 different couples out of about 155 individuals were recorded. Mating occurred when ladybirds accumulated a minimum of 18 ADD.

Table 1. Number of males with sperm cells in their testes, sperm cell number and viability during the two studied successive overwintering periods at the 3 studied sites. N = Number of sampled males.

Sites	Date	N	Males with sperm cells (%)	Sperm cell number (mean \pm SE)	Sperm cell viability (mean \pm SE, %)
<i>Year 1 (2013-2014)</i>					
LG	Nov.	45	86.7	165,867 \pm 91111	73.23 \pm 3.06
	Janv.	44	91	459,116 \pm 64733	84.51 \pm 2.72
	March	39	100	523,788 \pm 55685	80.48 \pm 4.03
MS	Nov.	41	78	131,183 \pm 70873	89.30 \pm 2.56
	Jan.	42	100	67,700 \pm 6189	84.26 \pm 1.88
	March	41	97.5	570,386 \pm 64061	85.52 \pm 2.41
	April	45	97.7	489,912 \pm 51284	72.48 \pm 3.78
<i>Year 2 (2014-2015)</i>					
LG	Nov.	41	100	431,141 \pm 35176	88.2 \pm 2.35
	Jan.	49	100	390,397 \pm 38797	88.2 \pm 1.11
	March	48	100	277,929 \pm 25201	92.5 \pm 1.09
MS	Nov.	41	100	421,745 \pm 35445	85.8 \pm 3.10
	Jan.	51	98	299,648 \pm 40137	90.5 \pm 1.60
	March	40	100	227,702 \pm 27766	90.5 \pm 1.85
	April	49	91.8	280,671 \pm 27638	91.8 \pm 1.50
SML	Nov.	20	100	416,705 \pm 53609	90.6 \pm 2.49
	Jan.	23	100	490,003 \pm 35349	77.4 \pm 2.16
	March	25	100	665,913 \pm 473465	91.4 \pm 1.22
	April	21	85.7	222,505 \pm 28728	91.9 \pm 2.65

a) 2013-2014



b) 2014-2015

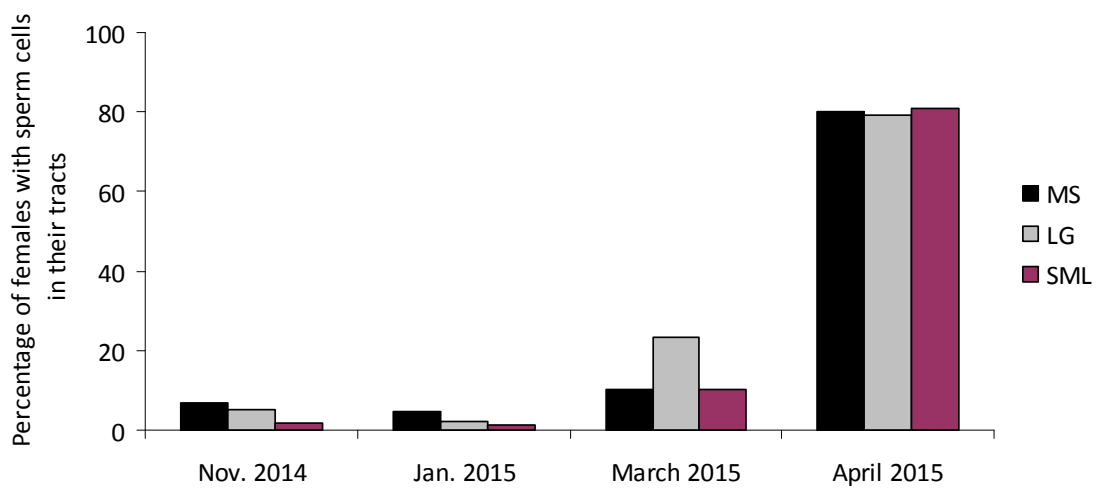


Figure 1. Percentage of females with sperm cells in their spermatheca and *bursa copulatrix* in two successive overwintering seasons. In 2013-2014, the aggregation at SML was destroyed in the beginning of March due to important rainfall; SML data were thus non available for April 2014. April 2014 and 2015: exhaustive sampling at LG.

Table 2. Female sperm cell number and viability during the two successive overwintering periods at the 3 study sites. N = Number of sampled females. Nmated = Number of females with sperm in spermatheca and *bursa copulatrix*.

Sites	Date	N	Nmated	Sperm cell number (mean \pm SE)	Sperm cells viability (mean \pm SE, %)
<i>Year 1(2013-2014)</i>					
LG	Nov.	43	4	4765 \pm 2808	71.2 \pm 15.9
	Jan.	42	2	6100 \pm 1910	68.48 \pm 15.48
	March	42	17	15186 \pm 2747	91.73 \pm 2.15
	April	124	113	8988 \pm 1222	86.85 \pm 2.58
MS	Nov.	35	1	100	100
	Jan.	42	1	2270	85.28
	March	45	7	14185 \pm 4161	74.24 \pm 12.96
	April	41	27	5720 \pm 542	84.04 \pm 3.12
<i>Year 2(2014-2015)</i>					
LG	Nov.	45	7	6714 \pm 3380	88.1 \pm 4.17
	Janv.	52	10	3200 \pm 753	91.7 \pm 5.14
	March	51	12	5440 \pm 2400	93.6 \pm 2.71
	April	47	39	3650 \pm 711	93.7 \pm 1.6
MS	Nov.	45	3	3517 \pm 1343	93.3 \pm 6.7
	Jan.	42	2	9250 \pm 8750	92.5 \pm 7.5
	March	50	4	6900 \pm 2866	91 \pm 3.87
	April	46	37	6222 \pm 832	92.9 \pm 1.15
SML	Nov.	20	1	3750	90.6
	Jan.	24	3	2417 \pm 843	89.02 \pm 5.48
	March	20	2	3500 \pm 2500	90 \pm 10
	April	25	19	7277 \pm 1541	92.5 \pm 2.01

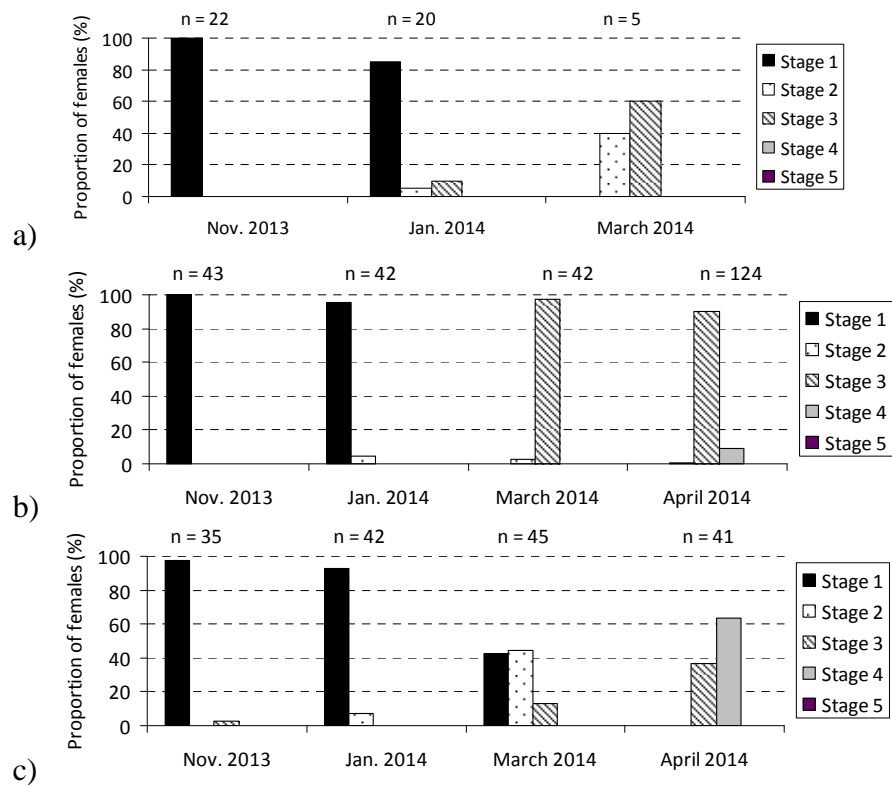


Figure 2 Stages of maturation of the gonads of *Hippodamia undecimnotata* according to the sampling locations and periods in the season 2013-2014 at a) SML, b) LG, and c) MS. In March 2014, only 5 females were sampled at SML due to site destruction.

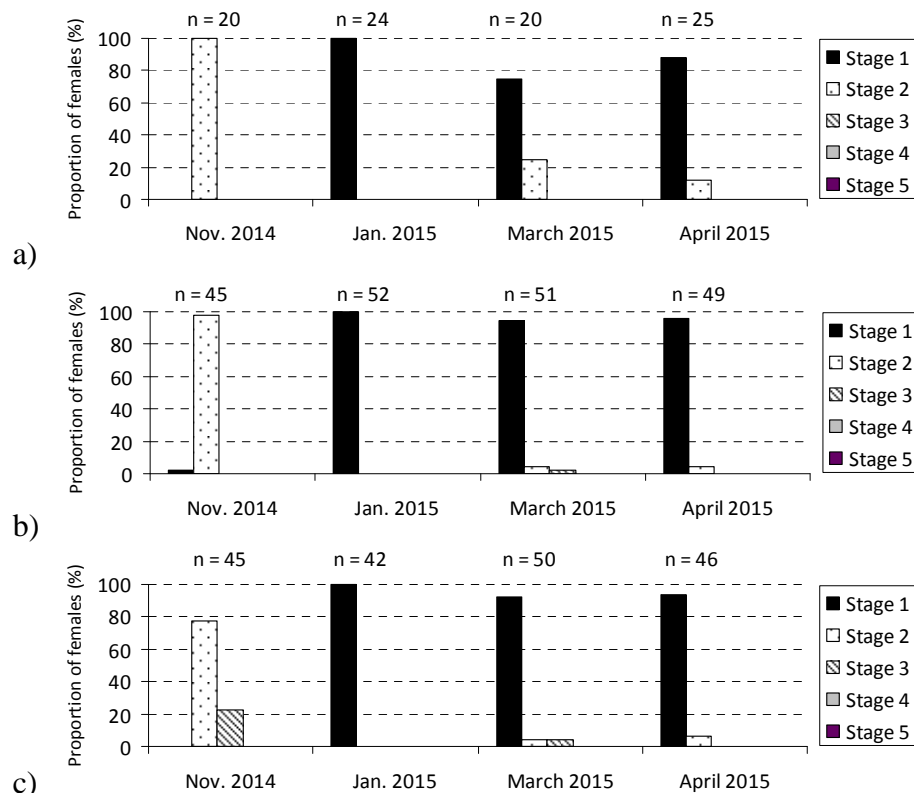


Figure 3 Stages of maturation of the gonads of *Hippodamia undecimnotata* according to the sampling locations and periods in the season 2014-2015 at a) SML, b) LG, and c) MS.

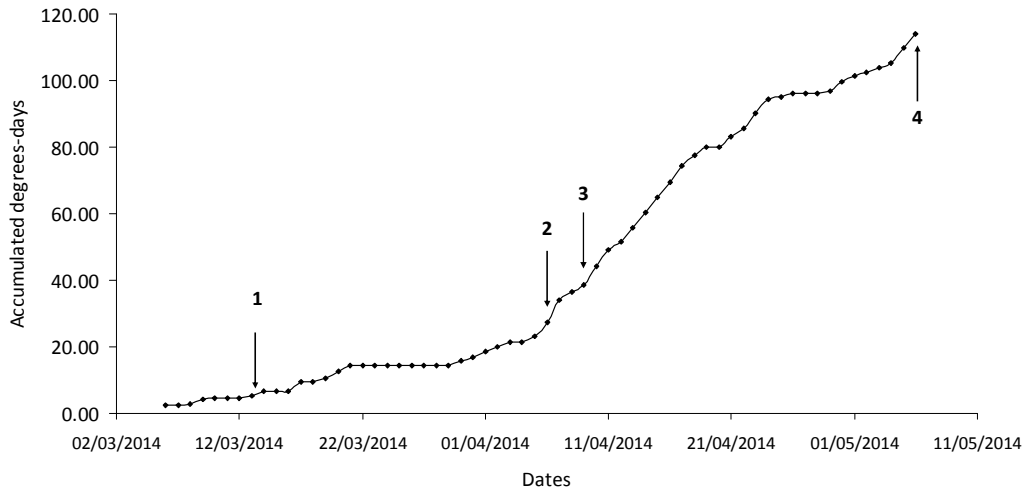


Figure 4 Accumulated degree-days by ladybirds at LG aggregation site in year 1.

1. Entomological trap set up; 2. First mating; 3. First dispersals; 4. Entomological trap removed.

DISCUSSION

The evolutionary significance of the diapause aggregations of arthropods is far from clear. We studied whether ladybird *H. undecimnotata* aggregations can be part of its mating strategy, as suggested by the hidden lek hypothesis of Wagner (1993, 1998). We investigated female mating status, at regular intervals, for two overwintering periods. We analyzed male and female gonads to provide insights into both sperm number and sperm viability. In parallel, we evaluated the ovarian status of females within aggregations. Furthermore, an exhaustive collection of females in one cluster gave us the proportion of mated females at the time of dispersal. Finally, mating behaviour was checked regularly during the overwintering period.

Most males at the aggregation sites had sperm cells

We found that 78% to 100% of the males had sperm cells in their testes for the whole of two consecutive overwintering periods (2013-2014 and 2014-2015). Males with sperm had high numbers (several hundreds of thousands) of highly viable sperm cells in their testes. Moreover, the percentage of males with sperm cells and the viability of these cells increased along the overwintering period, which matches with the observation that testes activity increased with day length and temperature, as observed in other species (Tanaka et al. 1987). Furthermore, the observation that the sperm cell numbers in testes decreased by between 15 to 70% just before dispersal also suggests that many mating events occurred at the aggregation before dispersal. As sperm production is energetically costly (Perry & Rowe 2013) males that had mated probably had no opportunity to renew their sperm stock at the aggregation site.

Females arrived with empty tracts but left loaded with sperm cells

Early during the overwintering period, most females had empty spermatheca and *bursa copulatrix*, indicating they were not mated. The less than 15% of them having sperm cells on arrival at aggregation probably resulted from the rare early copulations that we observed before aggregating. Alternatively, some of those females might have been mated during the previous overwintering period, seven months before, ladybirds being able to store sperm for several months (Awad et al. 2013). We cannot rule out that some *H. undecimnotata* survive the summer and thus participate to another aggregation, like other ladybirds (*Harmonia axyridis* (Pallas) or *Henosepilachna pustulosa* (Kôno); Savoiskaya 1970 in Awad et al. 2015; Nakamura & Ohgushi 1979; reviewed in Hodek et al. 2012). We found that the proportion of females with sperm cells, as well as their sperm cell number and quality increased along the winter, with the proportion of mated females reaching 65% to 91% depending of the cluster, when leaving the aggregation. Altogether our results strongly suggest that mating mainly takes place before leaving the aggregation, which is backed up by the high number of copulations observed towards the end of the overwintering period, as well as the observed diminution in sperm counts in male testes at the time of leaving the aggregation.

Mating was common at the aggregation sites toward the end of the aggregation period

Finally, we observed that mating frequently occurred at the aggregation sites during the second part of the overwintering period (March-April), before cluster breaks. That mating activity corresponded to the moment when both sperm cell number and viability in male gonads and female sperm storage and deposition organs were the highest. In agreement with this, we found that most females had immature ovaries during winter diapause, as previously observed (El Hariri 1966; Hodek & Landa 1971; Hemptinne & Naisse 1987; Ceryngier et al. 2004). Despite such immaturity, females mated at the aggregation sites, in agreement with previous observations (Hodek & Landa 1971; Tanaka et al. 1987). In some insect species, diapausing females are reluctant to mate (Krysan 1990), however our results suggest that ovarian maturation is not an essential condition for the onset of mating in coleopterans. Altogether, our results strongly support the hypothesis that overwintering aggregations are part of the mating strategy of *H. undecimnotata*.

Aggregation site features

It should also be noted that by aggregating during the overwintering period on the top of promontories, at the base of prominent features (Hagen 1962; Hodek 2012) and mating at those sites, *H. undecimnotata* behaves like hilltopping-displaying insects (Alcock 1987).

Hilltopping is a mate-locating strategy in which insects head to topographical summits or prominent features to meet their potential mates (Alcock 1987). The mating system of these insects is generally akin to a lek-system (Bradbury 1981; Alcock 1987). However, ladybird overwintering aggregations had never been considered before as a case of hilltopping. In hilltopping behaviour, the constancy of the aggregation sites and their prominent features, comparable to landmarks, are considered to ease mate finding (Cuddington et al. 2015). Some of the characteristics are fulfilled by ladybird overwintering aggregations. It remained to be investigated if the sex-ratio is biased at the beginning of the overwintering period (Capinera & Skevington 2008) to lead us to considered overwintering aggregation as a case of hilltopping.

Conclusion: are diapause aggregations sexually driven?

For decades, the traditional economic approach was used as a paradigm for the evolution of animal aggregation, and remained accepted uncritically. However, several authors have brought convincing arguments in favour of the role of mate choice in animal aggregation (Stamps 1988; Wagner et al. 2000). More generally, a few authors suggested that the origin of animal aggregations may rest in selection acting on the many processes of choice of all the commodities necessary to survive and breed, be they mates, food, breeding sites, shelters, ... rather than on direct advantages of aggregation (Danchin & Wagner 1997). Here we only dealt with one of these commodities, namely potential mates.

Concerning the role of sex, female choice behaviour was shown to lead to the formation of aggregations in the least flycatcher *Empidonax minimus* (Tarof et al. 2005), in the superb fairy-wren *Malarus cyaneus* (Cockburn et al. 2009), and in the shell-brooding cichlid fish *Laprologus callipterus* (Schütz et al. 2015). It was also suggested in the black-capped chickadee *Poecile atricapillus* (Danchin et al. 2008). The role of mate-finding strategies in the evolution of animal group living could therefore be a widespread phenomenon. Our results suggest that aggregations are part of the mating system of *H. undecimnotata* and that these aggregations may thus have evolved through benefits of mate-finding in many species. This is supported by the fact that several species such as butterflies (Lepidoptera), the fungus beetle *Stenotarsus rotundus* Arrow (Coleoptera, Endomychidae), or the kudzu bug *Megacopta cribraria* (F.) (Hemiptera: Plataspidae) have been documented to mate at the aggregation sites before dispersal (Monteith 1982; Wells & Wells 1992; see review in Majerus 1994 for ladybirds; Tanaka 2000; Golec & Hu 2015). According to this view, by aggregating, individuals would have the assurance of finding and even being able to

choose among potential mates (Kokko 1997; Hutchison 2005; Isvaran & Pongshe 2013) but the exact selective pressures driving them to join these sites remains unknown.

Alternatively, however, species might first have been forced to aggregate for other reason, and only then evolution favoured individuals able to use that opportunity to also find a mate. A way to disentangle these two exclusive alternative evolutionary pathways would be in using comparative approaches as this was done in birds (Rolland et al. 1998; Varela 2007). Nevertheless, before being able to adopt such an approach, we need to deepen our knowledge on mate choice behaviour and mating systems across many species of group-living insects. For instance, paternity analyses in two ladybird species showed that females are polyandrous (Haddrill et al. 2008; Awad et al. 2015) whereas patterns in males are unknown. These results and our findings about aggregation site features (Susset et al. submitted) suggest that ladybirds' aggregations may constitute a promiscuous mating in which females regularly mate multiply (Lanctot et al. 1997; Lank et al. 2002). In addition, the hidden lek hypothesis suggests that aggregation could be driven by sexual selection as a result of female preference for extra-pair copulations even in socially monogamous species (Wagner 1993, 1998; Fletcher & Miller 2006). This could explain why some females were found to be genetically polyandrous. Another possibility for this pattern is that the mating system of ladybirds is promiscuous, with both males and females mating without restrictions (Danchin et al. 2008). Studying these questions by detailed combining behavioural observations with paternity analyses is necessary to deepen our knowledge of the evolutionary mechanisms of insect overwintering aggregations and of the selective pressures shaping the mating strategy of aggregation-displaying insects in general.

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Mating during diapause aggregation: is it energetically costly?

In preparation



Photographie IV. Agrégation de coccinelles prise dans la glace (Mont Seigne, janvier 2015) ©Eline Susset

Mating during diapause aggregation: is it energetically costly?

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Abstract: Mating is a costly activity in animal life history and can sometimes lead to reduction in lifetime reproductive success and longevity. Surprisingly, for many species, mating occurs at the time of food deprivation, an environmental stressor related to seasonality. This is the case of many species forming diapause aggregations. Although energy consumption linked to food deprivation has been studied, few insights have been provided on the energetical costs of mating. In this study we investigated energy expended in mating during the diapause period in the aggregation-forming ladybird *Hippodamia undecimnotata* (Schneider). By quantifying energetic reserve amount before and after the mating period, we analyzed which energetic classes were used and whether reserves were depleted by mating activities. We also quantified the amount of remaining energetic reserves at the time of dispersal. Our results showed that ladybirds lost 3 to 14% of their weight during the mating period. Lipids and glycogen are the main energetic classes used during mating. During the mating period, males spent more energetic reserves than females. At the time of dispersal from the aggregation sites, ladybirds aggregating at the high altitude site bear a higher total energetic amount than ladybirds at low altitude site. Although mating incurs a loss of energy, it does not deplete energetic reserves neither in males nor in females. Ladybirds seem to manage their energetic consumption to be able to carry out the following step of the reproductive process and dispersal after aggregation period. How the use of energetic reserves during mating period affect the subsequent behaviour is discussed.

Introduction

For many organisms, mating events are costly and can even lead to reduction of lifetime reproductive success and longevity (Chapman et al. 1995; Kuijper et al. 2006). The costs of mating include increased rate of predation, injuries, disease transmission or energetic costs (Arnqvist 1997; Kotiaho et al. 1998; Watson et al. 1998; Blanckenhorn et al. 2002; Rolff & Siva-Jothy 2002). Energetic reserves at the time of mating affect reproductive success (Yuval et al. 1998). Indeed, individuals bearing more reserves generally have higher access to

mates because they are more competitive (Yuval et al. 1998; Shelly et al. 2002) but some exceptions exist (Maïga et al. 2014). Although costs of mating have been identified, energetic costs of mating have been barely quantified (Simmons et al. 1992; Maïga et al. 2014), more particularly in the context of prolonged food deprivation. However, determining the energetic costs of mating is of high importance as it can impact post food deprivation behaviour and life history (Warburg & Yuval 1997; Hahn & Denlinger 2011).

Many insects, such as the tropical beetle *Stenotarsus rotundus* Arrow (Wolda & Denlinger 1984) or the monarch butterfly *Danaus plexippus* L. (Tuskes & Brower 1978), form dense aggregations during the 5 to 8 months of the harsh season, when abiotic conditions are non suitable and food is unavailable. When arriving at the aggregations sites, arthropods are in diapause (Wolda & Denlinger 1984; Hodek 2012). Diapause is a genetic-determined state that allows the organisms to survive the unfavourable period by reducing physiological and metabolic functions, and thus by limiting energy use until suitable conditions are available again (Danks 1987; Košťál 2006). Nevertheless, diapause does not always last as long as the unfavourable season (Hodek 1996) and after a period of inactivity, activity is resumed and mating is observed (Hill et al. 1976; chapitre 3). Indeed, copulations occur at high rates few weeks before insects leave the diapause aggregation sites although individuals have been deprived from food for long (Hill et al. 1976; Taylor 1984; Tanaka et al. 1987; Hodek 2012; chapitre 3). For aggregation-displaying individuals, energetic expenses at the time of mating can come from courtship, producing gametes and ejaculates, inter-male competition for males, and from male carrying or mating avoidance for females (Watson et al. 1998; Perry & Tse 2013). After the aggregation period, individuals disperse and move to breeding sites; females will have to find suitable oviposition sites, and to produce and lay eggs (Dixon 2000), which is also energetically costly.

Insects' energy is stored as three major kinds of macronutrients: lipids, carbohydrates and proteins (Hahn & Denlinger 2011), with glycogen (a kind of carbohydrates) and lipids being the most abundant (Arrese & Soulages 2010). Glycogen is a fuelling catabolism, a precursor of cryoprotectant molecules such as glycerol and sorbitol, and is mobilized for egg production (Hahn & Denlinger 2007; Arrese & Soulages 2010). Lipids are used for the basal metabolism during diapause and play a role in reproductive physiology such as egg production and ejaculate composition (Zhou & Miesfeld 2009; Sloggett & Lorenz 2008; Hahn & Denlinger 2011). Among lipids, triglycerides mainly constitute the fat storage, and provide a water source upon oxidation (Arrese & Soulages 2010). Carbohydrates are sometimes converted to triglycerides and other lipids to renew fat storage, and compose the ejaculates in

males (Arrese & Soulages 2010; Perry et al. 2013). Proteins play a role in anabolic activities, catabolic respiratory metabolism, fuelling flight, and egg and ejaculate production (Hahn & Denlinger 2007; Foray et al. 2012). Proteins are also expected to play a role in post diapause tissue remodelling (Hahn & Denlinger 2007).

In this study, we investigated the physiological costs of mating and tested whether mating deplete energetic reserves in the ladybird *Hippodamia undecimnotata* (Schneider), a species known to form overwintering aggregations (Hodek 2012; Ceryngier 2015) and whose mating period happens few weeks before it leaves the aggregation sites (chapitre 3).

Ladybird mating behaviour includes mate recognition through contact infochemicals, courtship and copulation (Majerus 1994; Hemptinne et al. 1996). During copulation, males transfer to females an ejaculate mainly containing spermatozoa and seminal fluid composed of proteins, carbohydrates and lipids (Perry & Rowe 2008; Perry et al. 2013). These biological compounds are transferred in a spermatophore, mainly composed of proteins (Heller et al. 1998; Murphy & Krupke 2011). Therefore, we expect males to suffer from a depletion of their reserves after the time of mating, more particularly of lipids, proteins and carbohydrates which are the main components of ejaculates (Perry et al. 2013). By contrast, oocytes do not mature during the course of aggregation (Hodek & Landa 1971; Hemptinne & Naisse 1987; chapitre 3). The production of gametes in females is thus expected to occur after the aggregation period and should not impact the energetic reserves. Moreover, after the aggregation period, females will migrate back to oviposition sites and lay their eggs (Dixon 2000). We thus expect that mating leads to a reduction of energetic reserves in females through male carrying for example but this consumption should not lead to the reserve depletion of carbohydrates because it is a fuelling component of flight and it will be used for the return flight to the breeding sites (Amat et al. 2012) or lipids that in addition to glycogen will be used for egg production (Rankin & Burchsted 1992; Arrese & Soulages 2010).

Material & Methods

Ladybird sampling for energetic budget analyses

Ladybirds were sampled from 2 overwintering aggregation sites: a low altitude site, Labastide-Gabause (LG, Latitude: 44°2'N, Longitude: 2°6'E, Altitude: 260 m a.s.l.), and a high altitude site, Mont Seigne (MS, Latitude: 44°12'N, Longitude: 2°55'E, Altitude: 1128 m a.s.l.). Samplings were done just before mating started and before ladybirds dispersed from

the aggregation sites (i.e. April-May). Twenty males and twenty females were sampled at each sampling period, in each site.

The ladybirds were carefully collected with fine tweezers not to disturb the other ladybirds (making them move or fall) and put in a sampling box. After sampling, they were transported to the laboratory by means of a portative fridge at 10°C and were stored in a deep freezer (-80°C) at laboratory until required for energetic analyses.

Insect preparation and biochemical analyses of energetic budget

The ladybird corpses were dried in an oven at 35°C for 24h. Then they were weighed with a microbalance (Sartorius Supermicro) at the nearest 0.001 mg (initial dry mass). The width of the pronotum was measured because it is a good proxy of the overall body size of insects (Eberhard & Gutiérrez 1991). Each insect was individually placed into a 2 mL Eppendorf tube containing a stainless steel bead and 180 µL of aqueous lysis buffer solution [100 mM KH₂PO₄, 1 mM dithiothreitol (DTT) and 1 mM ethylenediaminetetraacetic acid (EDTA), pH 7.4] and was crushed by shaking the tube for 90 s at 25 Hz (Tissue Lyser; Qiagen, Valencia, California). We then quantified the complete energetic budget in the whole body of the ladybirds following Foray et al. (2012) method. This method allows the simultaneous quantification, in the same individual, of five major energetic components: proteins, carbohydrates (soluble carbohydrates “carbohydrates” and glycogen), and lipids (total lipids and triglycerides) (Figure 1). Some minor modifications have been made compared to Foray’s et al. (2012) method to adapt it to a ladybird as it is bigger and contain a higher cuticle surface than the biological model used by Foray et al. (2012). These modifications concerned the quantification of glycogen content. Instead of filtering the samples before reading the absorbance of the samples at 625 nm, we centrifuged them at 16.000 rpm during 30 min to make all the persistent cuticle debris agglomerated at the bottom of the Eppendorf tube.

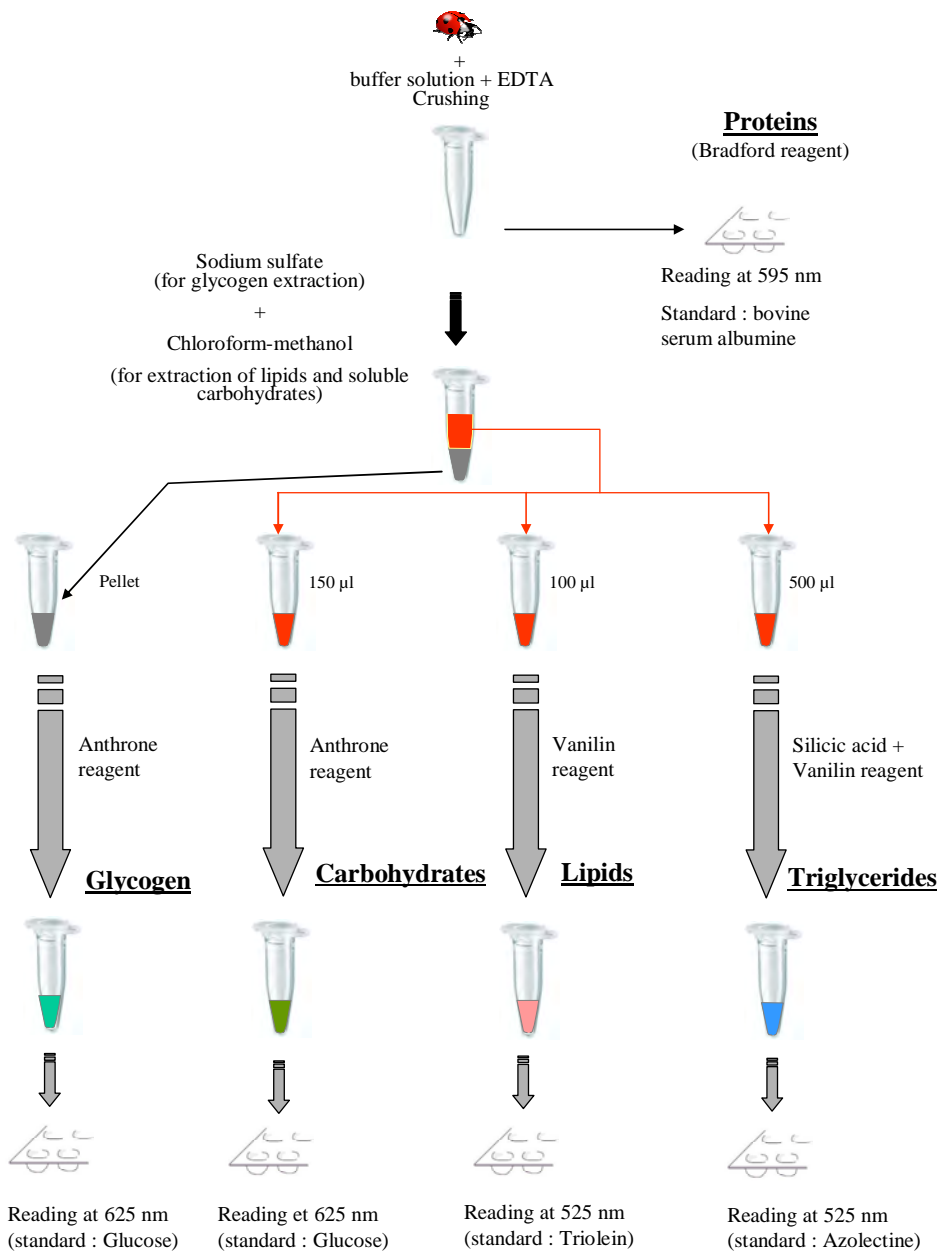


Figure 1. Successive steps of the method used to quantify the amounts of energetic reserves (proteins, glycogen, carbohydrates, lipids, triglycerides) in *Hippodamia undecimnotata* (adapted from Foray et al. 2012 by Felipe Ramon Portugal).

Environmental conditions at the aggregation sites

In addition, we compared abiotic conditions (mean daily temperatures) at the overwintering sites between September 1st, 2013 and May 10th, 2014. No meteorological records were available for the whole overwintering period at the exact places where ladybirds overwinter. So, we used data from the French national meteorological service database from the nearest sites: Montels for LG (18 km apart), and Salles-Curan for MS (14 km apart).

Statistical analyses

We used a generalized linear model (GLM) to analyse the effects of the sampling Period, Sex and their interactions on the weight controlled by the body size (ratio body mass/body size RMS) before and after the mating period. Then, we analyzed the effects of the sampling Period, Sex, and their interactions on the difference in each energetic class amount between before and after mating period by means of GLM with a gamma distribution (Foray et al. 2012). Differences between the mean daily temperatures at the overwintering sites (estimated by neighbour sites) were analyzed with a linear model with site and time (calendar dates) as explanatory variables. We compared the total amount of energetic reserves and the amount of each energetic class that remained in females and males at the end of the overwintering period with a linear regression. All analyses were carried out with the free software R 3.1.3 (R Development Core Team 2012).

Results

Before versus after mating difference

The energetic budget of ladybirds before the mating period was mainly composed of lipids (57 to 78% of total amount) and glycogen (8 to 20%), followed by proteins (9 to 17%), triglycerides (2 to 4%), and carbohydrates (1 to 4%). During the mating period, the RMS decreased by 16% and 3% for males and females at MS while it decreased by 14% for males and increased by 13% for females at LG. The energetic reserve analyses revealed that the amounts of proteins, carbohydrates, glycogen, and lipids were significantly different before and after mating in both sites for both sexes (Table 1). Protein amount decreased in ladybirds from both sites. Variations of carbohydrate and lipid amounts were site-dependent. Carbohydrate amount increased in both LG males and females whereas it decreased in both MS males and females (Figure 2). Lipid amount decreased in both LG males and females whereas it increased in MS males and females (Figure 2).

Concerning glycogen amount, it was significantly different before and after mating only at MS. Triglyceride amount did not change according to mating activities neither in males nor in females (Table 1).

Table 1. Summary of results for generalized linear models for proteins, carbohydrates, lipids, triglycerides, and glycogen concentrations. Data in bold indicate a significant effect.

a) Labastide-Gabausse

Effect	Proteins			Carbohydrates			Lipids			Triglycerides			Glycogen		
	d.f.	F	$P > \chi^2 $	d.f.	F	$P > \chi^2 $	d.f.	F	$P > \chi^2 $	d.f.	F	$P > \chi^2 $	d.f.	F	$P > \chi^2 $
Period	1	4.62	0.03	1	69.7	< 0.001	1	20.8	< 0.001	1	0.04	0.84	1	38.1	< 0.001
Sex	1	1.53	0.22	1	0.12	0.73	1	4.30	0.04	1	0.04	0.84	1	7.84	< 0.01
Period * Sex	1	0.04	0.83	1	0.11	0.74	1	1.86	0.17	1	0.05	0.31	1	0.10	0.75

b) Mont Seigne

Effect	Proteins			Carbohydrates			Lipids			Triglycerides			Glycogen		
	d.f.	F	$P > \chi^2 $	d.f.	F	$P > \chi^2 $	d.f.	F	$P > \chi^2 $	d.f.	F	$P > \chi^2 $	d.f.	F	$P > \chi^2 $
Period	1	13.9	< 0.001	1	35.7	< 0.001	1	78.1	< 0.001	1	0.01	0.93	1	0.02	0.89
Sex	1	2.25	0.13	1	2.18	0.14	1	4.87	0.02	1	0.96	0.33	1	1.38	0.24
Period * Sex	1	3.46	0.06	1	0.06	0.8	1	0.04	0.84	1	0.01	0.91	1	3.73	0.06

Females and males condition at the time of dispersal

No energetic class amount was found to be depleted at the time of dispersal neither in males nor in females (Figure 3). Lipids are still the main component of the total amount of reserves, representing 70 to 78% of the total remaining reserves. Remaining glycogen composed 10 to 15% of the total amount of reserves, followed by proteins (7 to 9%), triglycerides (1 to 4%), and carbohydrates (1 à 2%).

The comparison of the amount of remaining energetic reserves in females showed that MS ladybirds leave their overwintering site with a higher total amount of energetic reserves than LG ladybirds ($F_1 = 15.40$, $P < 0.001$). By focusing on each energetic class, we found that MS females had higher amount of proteins ($F_1 = 20.45$, $P < 0.001$), lipids ($F_1 = 7.31$, $P = 0.01$), and glycogen ($F_1 = 12.34$, $P < 0.01$), but a lower amount of carbohydrates ($F_1 = 33.32$, $P < 0.001$) and triglycerides ($F_1 = 6.42$, $P = 0.01$) compared to LG females.

In males, the total amount of remaining energetic reserves at the time of dispersal is significantly higher in MS males than in LG ones ($F_1 = 7.82$, $P < 0.01$). It appears that MS males leave their overwintering site with a higher amount of lipids ($F_1 = 7.31$, $P = 0.01$) but a less amount of carbohydrates ($F_1 = 26.44$, $P < 0.001$). No significant difference was found in the final amount of proteins ($F_1 = 0.0004$, $P = 0.98$), triglycerides ($F_1 = 2.70$, $P = 0.11$), and glycogen ($F_1 = 1.03$, $P = 0.31$).

Environmental conditions at the aggregation sites

Mean daily temperatures significantly varied across the time ($F_1 = 6.15$, $P = 0.01$) at both sites. Moreover, they were significantly higher at Montels (estimation for LG) than at Salles-Curan

(estimation for MS) ($F_1 = 67.79, P < 0.001$) (Figure 3). Time in interaction with site had no significant effect on mean daily temperature ($F_1 = 0.37, P = 0.53$).

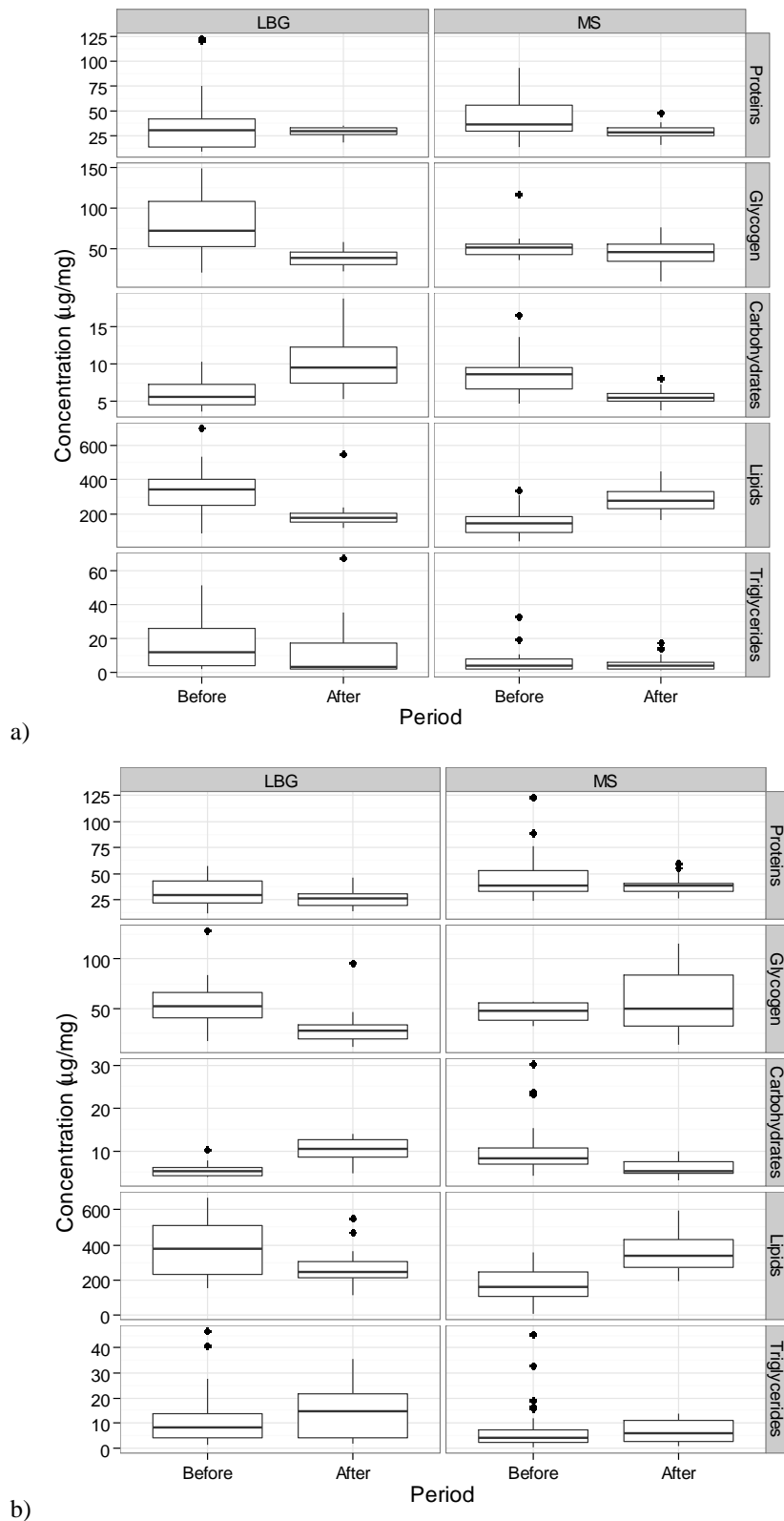


Figure 2. Dynamic of energetic resource consumption according to periods (before and after mating), sites and sexes. Amount of energetic reserves are given in μg of reserve class per mg of ladybird dry mass. a) Males, b) Females

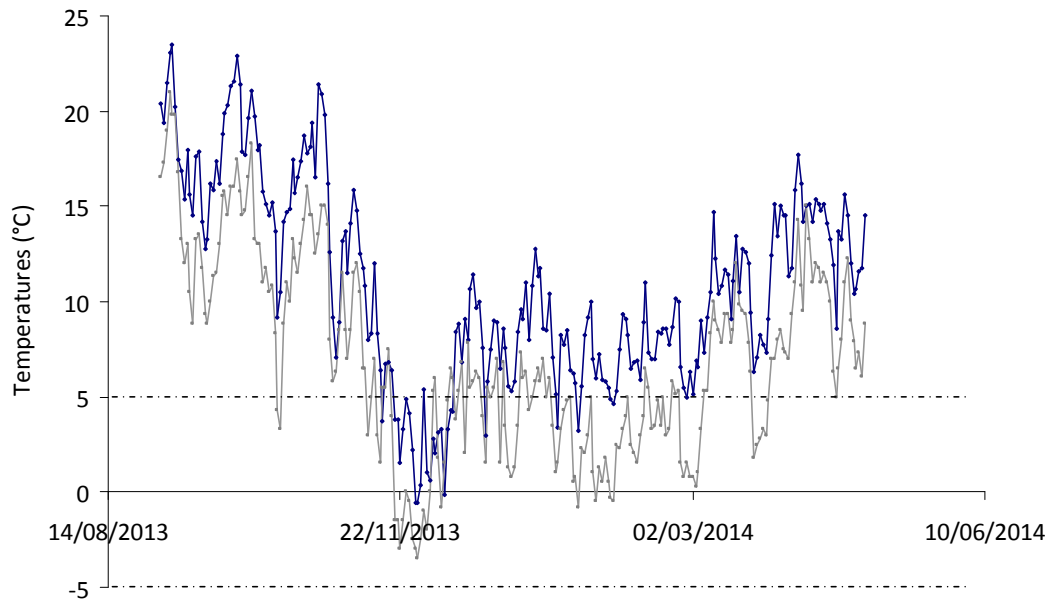


Figure 3. Mean daily thermal conditions at the overwintering sites (estimated from the nearest French meteorological station). For Mont Seigne (grey line): data from Salles-Curan station (14 km apart). For Labastide-Gabause (blue line): data from Montels station (18 km apart). Values between dotted lines correspond to optimal thermal conditions for ladybirds (i.e. energy consumption is low)

Discussion

The overwintering period is an ecological context in which insects in general, and ladybirds in particular, are affected by difficult abiotic conditions and are deprived from food, with no means to feed again during several months. For many arthropods it corresponds to the period of aggregation formation, which is a mating strategy to find mate and copulate (chapitre 3). The energetic reserves accumulated prior to food deprivation period and how these reserves are used are thus of tremendous importance because they allow an individual to survive and to reproduce. In this study we investigated the energetic cost of mating during diapause in the ladybird species *H. undecimnotata* and quantified each energetic class amount and the total amount of the remaining reserves at the time of dispersal. Our hypotheses were that 1) males should suffer from a depletion of their reserves after the time of mating, more particularly of lipids, proteins and carbohydrates, and 2) mating leads to a reduction of energetic reserves in females but this consumption should not lead to the reserve depletion. We found that ladybirds lost between 3% and 16% of their body mass corrected by body size (RMS) during the mating period, depending on the overwintering site where they form their aggregation. Our study identified lipids and glycogen, followed by proteins and carbohydrates, in LG ladybirds, and glycogen, followed by proteins and carbohydrates in MS ladybirds, as the main

energetic resources used during mating in *H. undecimnotata*. Changes in triglycerides' amount before and after the mating process seem to be negligible in both males and females as no significant difference was found between these two periods.

In males, the decrease of the energetic classes is attributable to the production of ejaculate and spermatophore, female courtship, and also basal metabolism (Watson et al. 1998; Hahn & Denlinger 2011; Perry & Tse 2013). Although a decrease of energetic reserves is expected due to male carrying during mating and basal metabolism, females are also expected to gain on weight during mating due to spermatophore transfer (e.g. Engqvist et al. 2014). Indeed, spermatophore is composed of a rigid capsule made of lipids and contains gametes but also a large amount of nutritional elements (Perry et al. 2013). In *Adalia bipunctata* (L.), the only ladybird in which spermatophore mass was investigated, ejaculate mass received by females during one copulation event can reach 4% of their body mass (Perry & Rowe 2010) but it can reach 7% of male body mass in other coleopteran species (Tallamy et al. 2000). Energetic reserves spent by females during mating (due to male carrying for example) could be compensated by the provision of spermatophores, suggesting that mating may provide energetic benefit to females. This could explain why mass loss in females is only 3% of RMS in MS and why we observed a gain on weight in females at LG. Surprisingly, there is an increase of the content of lipid between before and after mating in both sexes in MS ladybirds. In females, this could be due to spermatophore transfer as spermatophore is composed of lipids. In males, the question remains to be addressed.

At the time of dispersal, both males and females leave the aggregation sites with remaining reserves (about 40-70% of the initial total amount). We thus found that mating incurs a loss of energetic reserves in males and to a less extent in females, but it does not deplete the energetic reserves neither in males nor in females. Several studies have shown that ovaries are not mature at the time of dispersal (Hemptinne & Naisse 1987; Hodek & Landa 1971; chapitre 3). The delayed maturation of ovaries could be an adaptive strategy to avoid reserves depletion and keep reserves to flight towards breeding sites. From the male point of view, it seems that *H. undecimnotata* males have viable spermatozoa all along the aggregation period but the number of spermatozoa in the testicles increases through the aggregation period (chapitre 3). We expected that sperm production and resource allocation to ejaculate would deplete energetic reserves in males, in particular lipids and proteins which are the main components of spermatophore and its content (Perry et al. 2013). Contrary to our expectations, at the end of the aggregation period, males still had a high amount of energetic reserves allowing that they are able to flight back to the breeding sites, feed on aphids, and survive to

eventually keep mating. Contrary to the monarch butterfly (Oberhauser 1988), it seems that *H. undecimnotata* males did not mate until full reserve depletion but the reasons for this pattern remain to be investigated.

Interestingly, the comparison of the remaining amount of reserves at the end of the overwintering period taught us that ladybirds at the high-altitude site leave their overwintering site with a higher amount of reserves, even if they spent two more months overwintering. Both locations significantly differed from each other according to their abiotic conditions. The high-altitude site underwent less meteorological variations across the overwintering period compared to the low-altitude site, low temperatures occurred sooner and ladybirds were protected from temperature oscillations as they spent most of the overwintering period under the snow cover (chapitre 2). Temperature oscillations are known to induce changes in diapause intensity, leading metabolism to be never fully suppressed and insects being subjected to freeze-thaw cycles (Hahn & Denlinger 2011; Sinclair 2015). It is widely accepted that insects overwintering at higher latitudes and under cold temperatures benefit from a more stable range of temperatures over time and therefore, limit the consumption of energetic resources (Sinclair 2015; Williams et al. 2015). The tendency observed in this study confirms what had been previously observed for the seven-spotted ladybird *Coccinella septempunctata* L. overwintering at higher altitudes and that consumed fewer amounts of reserves (Zhou et al. 1995). Thus there should be a trade-off between spending reserves during the flight migration and during the aggregation period.

The current study shows that energetic reserves were used for mating activities in *H. undecimnotata*. The limitation of our study relies on the fact that we do not exactly know the life history of the sampled ladybirds and did not distinguish between mated and potentially non-mated ladybirds. Nevertheless, at the sampling time corresponding to the “after mating” period, the proportion of mated ladybirds is high (chapitre 3) and we assume that most of the sampled ladybirds have mated at least once. The amount of remaining reserves at the end of the aggregation period is expected to have important effects on post-diapause behaviour and more particularly on the reproductive success of ladybirds. Insects have an energy level sensing (Hahn & Denlinger 2011) and adapt their feeding or mating behaviour accordingly (Warburg & Yuval 1997). Indeed, we know that females deprived from food can be reluctant to remate (Perry et al. 2009). Moreover, males can be limited in the number of mating due to a depletion of components of seminal fluid (lipids, carbohydrates) (Reinhardt et al. 2011) or change the composition of their ejaculates and spermatophores (Ferkau & Fischer 2006; Perry & Tse 2013). An interesting aspect to be addressed is whether mating behaviour and

frequency are influenced by remaining amount of energetic reserves and to what extent it influences mating system.

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Discussion générale et perspectives

A travers le monde, de nombreuses espèces d'insectes forment des agrégations saisonnières au cours de leur diapause ([Introduction générale](#) [Tableau 1](#)). Malgré l'importante littérature qui leur est consacrée et leur caractère remarquable, les agrégations des arthropodes pendant la diapause restent un mystère sur le plan évolutif.

Dans cette thèse, j'ai cherché à répondre à 4 questions principales en étudiant les agrégations de diapause de l'espèce de coccinelle *Hippodamia undecimnotata* :

- quelles sont les caractéristiques des sites d'agrégation aux échelles locale et paysagère ? (**chapitre 1**)
- les coccinelles s'agrègent-elles dans les sites qui leur offrent des conditions abiotiques optimales de survie ? (**chapitre 2**)
- la formation des agrégations hivernales est-elle une stratégie pour trouver des partenaires sexuels et s'accoupler ? (**chapitre 3**)
- Quels sont les coûts énergétiques liés aux accouplements dans un contexte de privation de nourriture ? (**chapitre 4**)

Dans le chapitre 1, j'ai développé un modèle de distribution d'espèces (SDM, Elith & Leathwick 2009) adapté à la localisation des sites d'agrégation d'*H. undecimnotata* afin de 1) connaître les variables locales et paysagères qui sont corrélées avec la présence de sites d'agrégation, et 2) prédire les sites favorables à l'établissement d'agrégation d'*H. undecimnotata* dans des aires géographiques inexplorées. En partant de l'affirmation que *H. undecimnotata* s'agrège préférentiellement dans des sites situés sur des promontoires (Hagen 1962 ; Ipert 1966), j'ai mis en évidence que les sites d'agrégation d'*H. undecimnotata* se situent à la base d'objets proéminents, et dans des zones avec un faible risque d'être atteintes par les pulvérisations de pesticides (Susset et al. submitted). D'autre part, il ressort que l'abondance des coccinelles dans les sites est positivement corrélée à l'altitude du site.

Dans le chapitre 2, j'ai vérifié en analysant les conditions abiotiques au plus près des agrégations si les sites d'agrégation attirent les coccinelles car ils offrent des conditions propices à la survie. Mon travail a montré que dans les trois sites étudiés les conditions abiotiques ne sont pas favorables à la survie des coccinelles car 1) les conditions abiotiques fluctuent au cours des journées et entre les jours, entraînant ainsi une modulation du métabolisme, et 2) les conditions abiotiques (températures douces couplées à un fort taux d'humidité) sont favorables à la prolifération des champignons ectoparasites, ennemis naturels

létaux des coccinelles. D'autre part, les sites d'agrégation d'*H. undecimnotata* répertoriés dans le sud ouest de la France (chapitre 1) sont situés à des altitudes semblables à celles de deux des sites étudiés dans ce chapitre. Ils n'offriraient ainsi pas les conditions abiotiques optimales pour la survie des coccinelles mais cela reste à confirmer par des enregistrements spécifiques dans un plus grand nombre de sites. D'autre part, ayant pleine conscience du caractère préliminaire des résultats de ce chapitre, je recommande qu'une étude à plus long terme (sur plusieurs années) soit menée pour confirmer mes premières conclusions. Une comparaison des conditions abiotiques dans et en dehors des sites d'agrégation sera également nécessaire pour conclure définitivement sur la qualité des sites d'agrégation en termes de conditions abiotiques.

Dans le chapitre 3, mes recherches ont été consacrées au comportement d'accouplements dans les sites d'agrégation afin de caractériser l'importance des agrégations dans le système reproducteur de l'espèce étudiée. Le premier résultat est que les coccinelles s'accouplent dans les trois sites d'agrégation étudiés pendant deux années consécutives. Une majorité de femelles (entre 71 et 91%) quittent le site d'agrégation en ayant été fécondées. Le deuxième résultat est que les spermathèques (organe de stockage du sperme chez les femelles) et les bourses copulatrices (organe de dépôt des spermatozoïdes chez les femelles) contiennent un grand nombre de spermatozoïdes dont la viabilité est très élevée (84% à 94%). Le troisième résultat est que les accouplements ont lieu en fin de période d'agrégation, avant que les coccinelles ne dispersent. En conclusion, les accouplements ne sont pas des événements opportunistes mais un comportement recherché par les coccinelles.

Dans le chapitre 4 consacré à la consommation des réserves énergétiques lors de la période d'accouplements, j'ai mis en évidence que les lipides et le glycogène sont les deux principaux types de réserves utilisés. Les accouplements entraînent un coût énergétique chez les mâles et les femelles mais n'épuisent pas les réserves énergétiques. Au cours de la période d'agrégation, les coccinelles gardent de l'énergie pour supporter les coûts énergétiques lors de la période des accouplements mais également pour les comportements d'après agrégation tels que la recherche de sites de ponte, la production d'œufs et la ponte pour les femelles ou bien l'approvisionnement alimentaire pour les mâles.

Les résultats de cette thèse montrent que les agrégations ont un rôle dans la reproduction d'*H. undecimnotata* et qu'elles permettent aux individus de trouver leurs partenaires sexuels.

Pourquoi ce choix de sites d'agrégation ?

Les agrégations d'*H. undecimnotata* se trouvent sur des promontoires surmontés d'un objet visible à longue distance. La présence d'un repère visuel (objet proéminent) dans le site d'agrégation est essentielle pour l'établissement des agrégations d'*H. undecimnotata* (chapitre 1). Les agrégations hivernales d'*H. undecimnotata* semblent s'inscrire dans la lignée des stratégies de reproduction basées sur des « landmarks » où mâles et femelles se retrouvent autour de lieux de rendez-vous visuellement remarquables pour copuler (Thornhill & Alcock 1983). La stratégie des landmarks est très utilisée chez des espèces de diptères, de lépidoptères et d'hyménoptères : les individus utilisent la topographie du paysage et convergent vers les sites en relief. Le lek est souvent le système de reproduction de ces espèces (Alcock 1987). Le système de la copulation dans des landmarks a surtout évolué dans les espèces à faible densité ou bien lorsque les probabilités de rencontre de deux partenaires sexuels sont faibles en dehors des landmarks, ce qui permet de repérer le site de rencontre à longue distance (Scott 1968 ; Cuddington et al. 2015).

Les densités de coccinelles aphidiphages telles qu'*H. undecimnotata* sont faibles (moins de 1 individu/m²) en dehors de la période d'agrégation (Elliott & Kieckhefer 2000). D'autre part, les coccinelles sécrètent des molécules infochimiques de contact servant à la discrimination intersexuelle (Hemptinne et al. 1996 ; Hemptinne et al. 1998) mais pas de phéromones sexuelles. Elles sont donc limitées dans leurs modalités de rencontre d'un partenaire sexuel. Au travers de l'agrégation, les densités de coccinelles augmentent, avec des densités les plus hautes observées dans les sites aux plus hautes altitudes (chapitre 1). La stratégie du hilltopping/landmark peut avoir évolué comme stratégie de recherche active pour permettre aux coccinelles de se retrouver de manière plus efficace et plus certaine qu'un pari sur des rencontres au hasard dans les sites de ponte.

*Les accouplements d'*H. undecimnotata* peuvent-ils avoir lieu en dehors des sites d'agrégation ?*

La proportion de coccinelles s'accouplant dans les sites d'agrégation est très importante. Cependant, nous avons peu d'information sur la possibilité que des coccinelles s'accouplent

en dehors des sites d'agrégation. L'occurrence d'accouplements en dehors des sites d'agrégation reste donc une question essentielle pour entériner le rôle de la recherche du partenaire sexuel dans la formation des agrégations (**Perspective 1**). D'après les résultats du chapitre 4, mâles et femelles partent des sites d'agrégation en n'ayant pas utilisé toutes leurs réserves énergétiques. Ils semblent ainsi en mesure de regagner les sites de ponte et pourraient s'y accoupler. Néanmoins, pour des raisons encore inexplicables, la sex-ratio est fortement biaisée vers les femelles dans les sites de ponte (Awad et al. 2015), ce qui limite la probabilité d'accouplements en dehors des sites d'agrégation.

La difficulté pour répondre à ces questions provient d'un manque de connaissance sur la dynamique des populations de coccinelles et leur distribution spatiale en dehors de la période d'agrégation. Adapter les techniques de marquage d'insectes traditionnelles aux coccinelles représentent un défi : les hydrocarbures cuticulaires déposés sur les élytres rendent éphémères les marques au stylo ou à la peinture. Par ailleurs, la stratégie « Capture-marquage-recapture » est difficile à mettre en œuvre car les coccinelles peuvent se déplacer sur de longues distances (Hodek et al. 1993) et elle ne permet pas le suivi précis et sur une longue période. L'inexistence de techniques de marquage non invasives durables ou de technologie de suivi GPS adapté à la taille des coccinelles telles que celles développées pour les fourmis (Moreau et al. 2011) ou pour les abeilles (Henry et al. 2012) accentue ce défi. En parvenant à lever ce verrou technique, de nombreuses questions passionnantes pourraient trouver des réponses et nous pourrions ainsi améliorer nos connaissances sur la dynamique spatiale et temporelle des populations et la valeur adaptative des agrégations hivernales. Une solution pourrait être de tester ces questions chez d'autres espèces (en dehors de la famille des Coccinellidae) pour lesquelles le problème de marquage n'existe pas.

*Les agrégations de diapause d'*H. undecimnotata* sont-elles des leks cachés ?*

Les résultats de cette thèse montrent que les agrégations de diapause sont liées au système de reproduction des coccinelles. Il reste maintenant à déterminer à quel régime d'appariement elles correspondent. Des analyses génétiques de paternité réalisées chez deux espèces de coccinelles multivoltines, *Adalia bipunctata* L. et *Harmonia axyridis* (Pallas), ont montré que toutes les deux sont polyandres (Haddrill et al. 2008 ; Awad et al. 2015). La polyandrie (une femelle s'accouple avec plusieurs mâles ; un mâle s'accouple avec une seule femelle) et la monogamie (mâles et femelles s'accouplent avec un seul partenaire pendant la saison de reproduction) *sensus stricto* sont des régimes d'appariement rares dans le règne animal

(Danchin et al. 2008) et ne devraient pas concerner les agrégations de diapause compte-tenu des résultats obtenus chez *A. bipunctata* et *H. axyridis*. En revanche, d'autres systèmes tels que les leks, les leks cachés ou la promiscuité sexuelle sont plausibles.

Les leks sont des agrégations de territoires de parade correspondant à un régime d'appariement polygyne : un mâle se reproduit avec plusieurs femelles tandis qu'une femelle ne se reproduit qu'avec un seul mâle (Danchin et al. 2008). De plus, le taux de paternité multiple est faible au motif que les femelles ont le temps de choisir leur mâle, préfèrent les mâles les plus attractifs et que ces derniers sont disponibles pour toutes les femelles (Westneat et al. 1990 in Lank et al. 2002). De ce fait, les espèces formant des leks sont considérées comme ayant de faibles taux d'accouplements : seul(s) un ou deux mâles obtiennent la majorité des accouplements (Bradbury 1981 ; Danchin et al. 2008 ; Lank et al. 2002 ; Danchin et al. 2008). Cependant, les accouplements multiples et la paternité multiple sont plus fréquents qu'attendus (Lanctot et al. 1997 ; Lank et al. 2002). Cette nouvelle caractéristique ouvre la possibilité que les agrégations correspondent à un système tel que les leks au moins chez les 2 espèces de coccinelles étudiées.

Par ailleurs, bien que similaires aux leks par leurs mécanismes de formation, les leks cachés sont des systèmes d'appariement observés chez des espèces considérées comme socialement monogames ou polygyne, mais au sein desquelles les femelles recherchent des copulations extra-paires (Fletcher & Miller 2006 ; Schütz et al. 2016). Les individus sont répartis sur un territoire plus large que dans le cas d'un lek car les territoires des mâles sont plus larges (Fletcher & Miller 2006). Chez plusieurs espèces d'insectes formant des agrégations de diapause, il est fréquent d'observer différents groupes d'individus au sein du site d'agrégation. Cette répartition spatiale des individus pourrait être considérée comme une forme de lek ou bien de lek caché. Dans les 2 cas, on s'attendrait à observer une situation dans laquelle les femelles s'accouplent avec plusieurs mâles et un déséquilibre du succès reproducteur entre les mâles (les mâles de grande qualité fécondant plus de femelles que les mâles de faible qualité).

Un troisième régime d'appariement plausible est la promiscuité sexuelle. C'est un régime d'appariement répandu chez les animaux dans lequel mâles et femelles s'accouplent avec plusieurs partenaires sexuels au cours d'une saison de reproduction (Danchin et al. 2008). Les analyses génétiques réalisées chez deux espèces de coccinelles montrent que les femelles sont capables de s'accoupler avec plusieurs partenaires (Haddrill et al. 2008 ; Awad et al. 2015). La polyandrie *sensu stricto* est un régime d'appariement très rare dans le règne animal (Danchin et al. 2008) et il est peu probable que les agrégations de diapause y

correspondent. Des analyses génétiques pour connaître le profil des mâles et identifier leur contribution relative des mâles dans la fécondation des femelles permettraient de savoir si les mâles s'accouplent avec plusieurs partenaires et ainsi savoir si la promiscuité sexuelle est le régime d'appariement dans les agrégations de diapause.

En l'état actuel des connaissances, il est difficile de savoir à quel régime d'appariement correspondent les agrégations de diapause. Bien que les deux espèces de coccinelles dont la polyandrie a été mise en évidence génétiquement forment des agrégations de diapause, les auteurs ne précisent pas si les coccinelles se sont accouplées dans le cadre des agrégations de diapause. De plus, il est difficile de généraliser cette hypothèse résultats obtenus chez seulement deux espèces à l'ensemble des espèces de coccinelles. Compte-tenu de la diversité des régimes d'appariement, il est nécessaire d'étudier la question du régime d'appariement dans le cadre des agrégations de diapause en réalisant des suivis comportementaux couplés à des analyses de paternité pour savoir à quel régime d'appariement les agrégations de diapause correspondent et comment la sélection sexuelle a contribué à leur évolution (**Perspective 2**).

Par ailleurs, un concept central lié à l'hypothèse du lek caché est l'utilisation de l'information publique qui influence la décision de rester sur un site ou de partir pour en trouver un autre (Danchin et al. 2008). Chez les oiseaux, qui peuvent vivre plusieurs saisons de reproduction au cours de leur vie, l'information publique est fournie par l'observation répétée du succès reproducteur des congénères (Doligez et al. 2002). Récemment, l'utilisation de l'information publique a été confirmée chez les invertébrés (Mery et al. 2009). Chez les coccinelles, l'information publique pourrait provenir des molécules infochimiques telles que les hydrocarbures cuticulaires ou les caroténoïdes, qui influencent un grand nombre de leurs comportements (Bezzares et al. 2007 ; Hemptinne & Dixon 2000 ; Bell & Cardé 2013). De nombreuses preuves se sont accumulées ces 5 dernières années montrant que la formation des agrégations hivernales est menée par des signaux conspécifiques (Durieux et al. 2013; Susset et al. 2013; Wheeler & Cardé 2013, 2014). Il reste à tester si ces signaux indiquent la qualité des individus présents, renseignent sur leur fitness et influencent la décision de rester dans un site ou d'en partir (**Perspective 3**).

Protéger les sites d'agrégation : un impact pour la lutte biologique ?

Dans la publication basée sur le chapitre 1 de cette thèse, nous attirons l'attention sur le fait que les sites d'agrégation à travers le règne animal sont des outils de conservation clés car ils

permettent de protéger un grand nombre d'individus sur une aire géographique réduite par rapport à la totalité de l'habitat (Dobson & Poole 1998). A la lumière des résultats de cette thèse, les sites d'agrégation de coccinelles (et potentiellement d'autres espèces) sont des lieux stratégiques pour leur reproduction et ainsi pour le renouvellement des communautés d'ennemis naturels.

La lutte biologique par conservation, définie par Eilenberg et al. (2001) comme « la modification de l'environnement ou des pratiques existantes pour protéger et favoriser les ennemis naturels afin de réduire les effets des insectes ravageurs » se développe de plus en plus et cherche maintenant à optimiser l'effet des ennemis naturels au printemps et en été en intervenant sur la phase hivernale de leur cycle de vie (Griffiths et al. 2008). Une perspective appliquée de ce travail pourrait être de quantifier l'importance et la qualité du réseau de sites d'agrégation sur l'efficacité de la lutte biologique et d'améliorer la qualité des sites d'agrégation, notamment en réduisant les perturbations anthropiques (**Perspective 4**). Par ailleurs, les coccinelles sont des animaux charismatiques, symbole de bonheur pour petits et grands et partenaires redoutables dans la lutte biologique (Majerus 1994 ; Diehl et al. 2013). Elles peuvent être un point d'entrée pour encourager le développement des mesures de protection des sites d'agrégations des ennemis naturels et attirer l'attention sur d'autres espèces jugées moins attirantes mais toutes aussi importantes dans le fonctionnement des écosystèmes.

L'histoire ne fait que commencer !



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Au cours d'une journée sur le terrain, je découvre deux coccinelles accouplées, cachées sous l'écorce d'un genêt. Quelques secondes après, plusieurs mâles arrivent de sous l'écorce et tentent violemment d'éjecter le mâle accouplé pour lui prendre sa place. La scène n'a duré que quelques secondes mais elle fut intense.

Cette photographie met en lumière la compétition qui existe dans les agrégations hivernales et annonce les perspectives passionnantes en termes d'écologies comportementale et évolutive offertes par l'étude des agrégations de diapause.

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TITLE: Importance of diapause aggregation in the reproduction of ladybird *Hippodamia undecimnotata* (Schneider) (Coleoptera: Coccinellidae)

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PLACE AND DATE OF PhD DEFENCE:

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ABSTRACT:

Many animals live in groups during part or their whole life. While the evolutionary significance of some kinds of group living are well-known, some others remain poorly known although widespread. A good example is given by the diapause aggregations in terrestrial arthropods, which are monospecific clusters formed at the same places year after. In this thesis, I tested if the arthropods form diapause aggregations to find their mates according to the hidden lek hypothesis with the ladybird *Hippodamia undecimnotata*. By using a Species Distribution Model, I showed that ladybirds aggregate in places with a prominent object and where the risk of being sprayed by pesticides is low. Then, I highlight that abiotic conditions at the nearest of the aggregations are unfavourable to ladybirds' survival. In a third part, I found that mating is widespread activity in the aggregation sites. Finally, I showed that energetic costs linked to mating are low, and thus allow the ladybirds to disperse and display post aggregations behaviour. This thesis shows that the diapause aggregations are part of the mating system of the arthropods and that sexual selection can be a driver of the evolution of diapause aggregations.

KEY-WORDS: Coccinellidae, aggregation, diapause, reproduction, energetic reserves, abiotic conditions, SDM, lek

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TITRE : Importance des agrégations de diapause dans la reproduction de la coccinelle *Hippodamia undecimnotata* (Coleoptera : Coccinellidae)

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RÉSUMÉ :

Au cours de leur vie, de nombreux animaux expérimentent la vie en groupe. Si les causes évolutives de certains groupes sont bien identifiées, ce n'est pas le cas de tous, et notamment des agrégations de diapause des arthropodes terrestres (rassemblements monospécifiques d'adultes dans des sites à localisation constante pendant plusieurs mois consécutifs). Dans cette thèse, j'ai testé si, selon l'hypothèse du lek caché, les arthropodes se rassemblent pour trouver leurs partenaires sexuels chez la coccinelle *Hippodamia undecimnotata*. A l'aide d'un modèle de distribution d'espèces, j'ai montré que les coccinelles se rassemblent dans des endroits avec un repère proéminent et où le risque de pesticides est faible. Puis j'ai mis en évidence que les conditions abiotiques dans les sites d'agrégations sont défavorables à la survie des coccinelles. Dans un troisième temps, j'ai mis en évidence l'occurrence de nombreux accouplements dans les sites d'agrégation. Enfin, j'ai montré que ces accouplements sont peu coûteux en énergie pour les femelles et permettent aux coccinelles de conserver de l'énergie pour les comportements post- agrégation. Cette thèse atteste que les agrégations de diapause font partie du système de reproduction des arthropodes et que la recherche de partenaires sexuels peut être l'un des moteurs de leur formation.

MOTS-CLES : Coccinellidae, agrégation, diapause, reproduction, réserves énergétiques, conditions abiotiques, SDM, lek

DISCIPLINE : Écologie, Biodiversité & Évolution

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