Evolution des stratégies de reproduction de parasitoïdes de drosophiles en réponse au climat

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Chapitre 1.

Introduction générale

Introduction générale

Prédire l'évolution des histoires de vie des organismes en réponse au réchauffement climatique global est devenu un enjeu majeur en écologie évolutive. Néanmoins, pour prédire cette évolution, il faut comprendre comment ces histoires de vie ont évolué et évoluent en réponse aux facteurs climatiques. Diverses approches ont été développées pour comprendre cette évolution. La première repose sur des mesures en laboratoire de la plasticité phénotypique des traits d'histoire de vie face à différents régimes thermiques. Les études clinales consistent quant à elles à prélever des populations le long d'un gradient climatique puis à les comparer en les élevant en conditions standard en laboratoire. On considère que les adaptations locales ainsi observées sont le résultat de la sélection par le climat d'origine. Les travaux de « sélection naturelle en laboratoire » permettent pour leur part de suivre l'évolution des traits mesurés à différentes températures. Plus récemment, l'essor de la modélisation en biologie évolutive a donné naissance à divers modèles permettant de prédire l'évolution des histoires de vie des organismes en réponse à des scenarios de modifications du climat.

Dans cette thèse, je me suis essentiellement intéressé aux adaptations locales des traits d'histoire de vie de parasitoïdes de drosophiles et de leurs normes de réaction, en intégrant deux approches : des études de variations géographiques des histoires de vie et des mesures de plasticité phénotypique en réponse à la température. Je présente dans la première partie de ce travail les différentes théories et éléments de l'écologie évolutive nécessaires à la compréhension de ces approches. J'aborderai dans une seconde partie le modèle biologique étudié, à savoir les insectes parasitoïdes. Les résultats majeurs obtenus durant la thèse seront ensuite présentés sous la forme de cinq publications, suivis d'une discussion générale de l'ensemble de ces résultats.

1- Histoires de vie et traits d'histoires de vie

A- Histoires de vie et trade-offs: la théorie

a) Définitions

Le terme d' « histoire de vie » fut défini pour la première fois par Stearns en 1976 comme « a set of coadapted traits designed, by natural selection, to solve particular ecologic problems ». Dans leur synthèse sur l'importance de la physiologie dans les histoires de vie, Ricklefs & Wikelski (2002) définissent ce terme par une combinaison de stratégies évoluées, incluant des adaptations comportementales, physiologiques et anatomiques, qui influencent plus ou moins directement la survie et le succès reproducteur.

Les traits d'histoire de vie sont donc des caractères liés à la fitness, comme la taille et l'âge à la première reproduction, la taille de ponte, le sex ratio dans la descendance ou encore la fécondité et la longévité. Par extension, d'autres caractères qui vont avoir une influence sur ces traits d'histoire de vie, comme la taille ou le taux de métabolisme, peuvent être considérés comme ayant une influence sur la fitness et sont alors parfois intégrés aux traits d'histoire de vie.

Sous l'impulsion de Stephen Stearns et de Derek Roff, de nombreux travaux se sont intéressés à la compréhension de l'évolution des histoires de vie, un thème central en écologie évolutive. Cette théorie de l'évolution des histoires de vie repose essentiellement sur un fait majeur : l'existence de contraintes. Pour tout organisme, la fitness optimale serait celle qui résulterait d'une maximisation de tous les traits d'histoire de vie dans l'environnement dans lequel celui-ci se développe. Néanmoins, l'augmentation de fitness associée à un changement dans un trait est souvent contrebalancée par une diminution de fitness associée à un changement dans un autre trait, on parle alors de trade-off, ou compromis évolutif. Il existe ainsi toute une gamme de combinaisons possibles de traits d'histoire de vie, dont l'une d'entre elles au moins sera supérieure aux autres dans un environnement particulier. C'est celle-ci qui devrait être sélectionnée.

« Selection does not produce perfect genotypes, but it favors the best which the numerous constraints upon it allow." (*Mayr 1983*)

Les mécanismes à l'origine des trade-offs peuvent être de deux types. Le premier est d'origine génétique : deux caractères sont gouvernés par le ou les mêmes gènes et l'expression d'un trait pourra altérer ou contraindre l'expression de l'autre. On parle alors d'effets pléiotropiques antagonistes **(**Roff & Fairbairn 2007). D'autres auteurs considèrent que les contraintes physiologiques peuvent être à l'origine de trade-offs (Calow 1979): la quantité de ressources énergétiques de l'organisme étant limitée, si davantage de ressources sont allouées à un trait, ceci se fera au détriment d'un autre trait.

L'existence de ces deux types de contraintes, génétiques et énergétiques, a amené de Jong & van Noordwijk (1992) à les considérer simultanément pour établir leur modèle en Y (Figure 1), repris depuis dans de nombreuses études. Dans ce modèle, l'acquisition et l'allocation de ressources sont chacune gouvernées par un seul et même locus et il existe une réserve commune de ressources à allouer à deux traits en compétition.

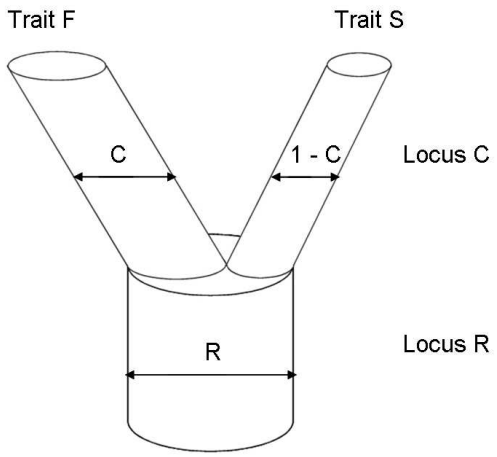

Figure 1. Modèle simple en Y décrit par de Jong & van Noordwijk (1992). La quantité limitée de ressources R, dont l'acquisition est contrôlée par le locus R, est allouée par le locus C aux deux traits F et S.

Les modèles théoriques plus récents considèrent que le pool de réserves énergétiques est compartimenté. Ainsi, des nutriments différents sont alloués aux différentes fonctions en compétition (pour une synthèse, voir Pelosse 2008). Cette allocation des nutriments entre les différentes fonctions est discutée plus précisément pour les insectes en partie 4b.

Récemment, le rôle de mécanismes hormonaux dans l'expression de trade-offs a été mis en évidence (Harshman & Zera 2006). Cette régulation hormonale est discutée dans la partie suivante.

b) Des trade-offs fondamentaux : les coûts de la reproduction

De part son importance dans la fitness d'un organisme, les traits et trade-offs liés à la reproduction ont reçu plus d'attention que n'importe quels autres traits. Lack fut le premier à observer en 1954 que la taille optimale de ponte des oiseaux dépendait de la quantité de ressources alimentaires dont les parents disposent pour nourrir leurs jeunes. C'est néanmoins Williams qui, en 1966, évoqua pour la première fois l'idée que l'effort reproducteur, parce qu'il est couteux en énergie pour l'individu, pouvait avoir des conséquences sur d'autres traits. Il montra ainsi, et ce constat fut confirmé par de nombreuses autres études ensuite, que la reproduction courante pouvait avoir un impact sur la reproduction future (Tinbergen 1987, Jervis & Ferns 2004). L'allocation optimale des ressources dans la reproduction serait donc déterminée par un équilibre entre les bénéfices à un temps t d'un évènement reproducteur (production d'œufs pour une femelle) et les coûts sur les reproductions futures. Cette idée de « coût de la reproduction » soulevée par Williams a donné lieu par la suite à d'innombrables travaux qui ont décrits d'autres coûts que celui sur la reproduction future, comme un coût sur la survie des individus (Stearns 1989). Ellers *et al.* (2000), en manipulant la production d'œufs de femelles de l'hyménoptère parasitoïde *Asobara tabida*, ont pu mettre en évidence ce trade-off entre fécondité et longévité. D'autres manipulations expérimentales de l'effort reproducteur ont montré des résultats similaires chez des reptiles (Shine & Schwarzkopf 1992), oiseaux (Nur 1988) et arthropodes (Bell & Koufopanou 1986, Creighton *et al.* 2009).

Calow (1979) estime que ces coûts sur la reproduction future et sur la longévité sont les deux grands coûts de la reproduction. Pourtant, l'investissement de ressources dans la reproduction peut induire d'autres changements de traits comme une diminution du stock des réserves énergétiques (Reznick 1983, Madsen & Shine 1993) et des capacités de vol et de dispersion (Zhao & Zera 2006), ou encore une limitation de la croissance du reproducteur (Cox & Calsbeek 2010, Reekie & Bazzaz 1992, Roff 1983). De plus, les contraintes énergétiques et mécaniques induisent des corrélations

négatives entre nombre et taille des œufs (Berrigan 1991, Fischer & Fiedler 2001, Reading 1986), cette dernière étant corrélée à la survie des descendants (Fischer *et al.* 2003, Roff 1992). Ces mêmes contraintes mécaniques impliquent que la gravidité peut être associée à une réduction des capacités locomotrices des femelles (Kullberg *et al.* 2002, Seigel *et al.* 1987, Shine 2003), augmentant ainsi les risques d'être prédatées (Pavlova *et al.* 2010, Lewis & Loch-Mally 2010). Ces coûts de la reproduction et corrélations entre fécondité et d'autres traits suggèrent que l'effort reproducteur ne peut être maximisé sans incidence sur d'autres fonctions de l'organisme, et donc que certaines stratégies reproductrices devraient être sélectionnées dans des environnements donnés. Travailler sur l'évolution des stratégies de reproduction exige donc d'intégrer autant que possible ces différentes composantes, en fonction de l'organisme étudié.

Comme discuté dans la partie précédente sur les trade-offs, les coûts de la reproduction sont associés à des contraintes physiologiques entre traits, via une quantité de ressources limitées, et à des effets pléiotropiques antagonistes (Zera 2005). Plusieurs auteurs ont mis en évidence que ces coûts de la reproduction pouvaient également être contrôlés par une régulation hormonale (pour une synthèse, voir Harshman & Zera 2006). Celle-ci implique par exemple chez la drosophile l'hormone juvénile, qui contrôle la production d'œufs. (Tatar *et al.* 2001). Dans cette étude, illustrée en Figure 2, une défaillance dans la production d'insuline entraine une diminution de la quantité d'hormone juvénile, qui entraine à son tour une diminution de la production d'œufs. Les auteurs observent alors une augmentation de la longévité, induite par les contraintes génétiques et physiologiques existant entre ce trait et la fécondité. Dans le cas contraire, une augmentation de la concentration en hormone juvénile et donc de la production d'œufs peut affecter le système immunitaire (McKean & Nunney 2005) et la résistance aux stress (Salmon *et al.* 2001, Alonso-Alvarez *et al.* 2004) du fait d'effets pléiotropiques antagonistes entre ces traits. Des contraintes énergétiques et une régulation hormonale ne se rejettent pas mutuellement, l'allocation des ressources pouvant être contrôlée par cette régulation hormonale.

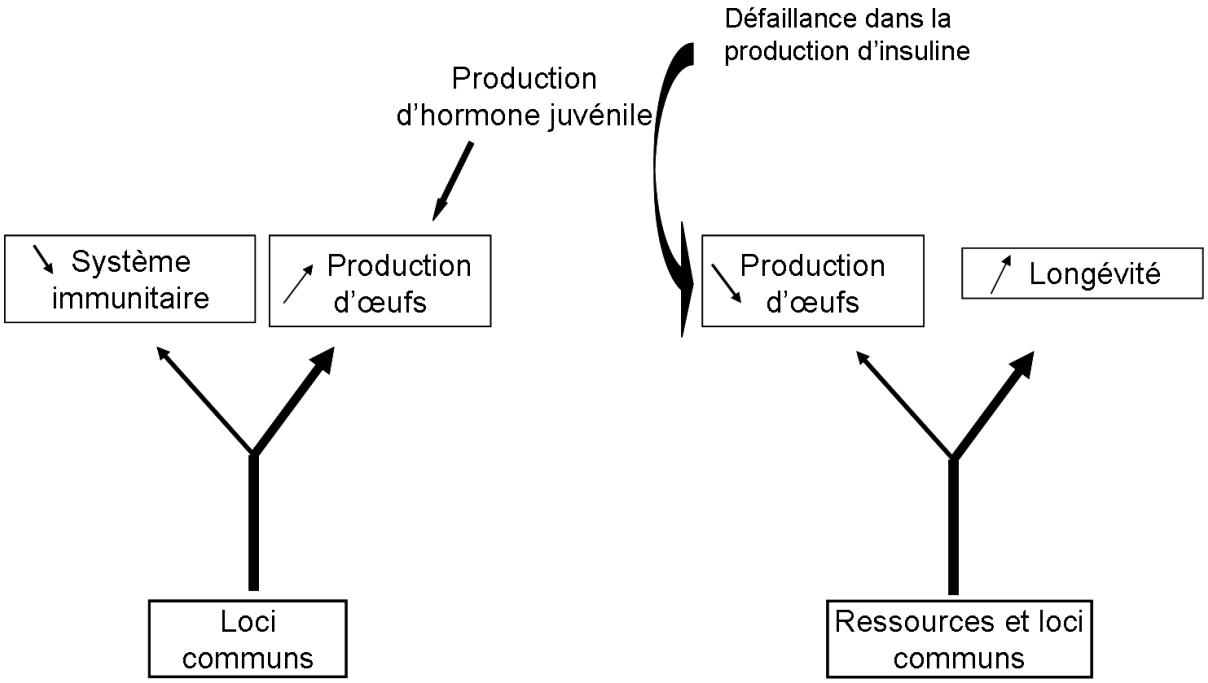

Figure 2. Régulation hormonale des traits d'histoire de vie. Une défaillance dans la production d'insuline entraîne une diminution de l'hormone juvénile (jh), causant une diminution de la production d'œufs, associée à une augmentation de la longévité. Une augmentation de la production de jh entraîne une augmentation de la production d'œufs, associée à une défaillance du système immunitaire.

B- Histoires de vie et trade-offs : la pratique

a) Evolution des histoires de vie

Il existe deux approches majeures pour comprendre l'évolution des histoires de vie (Roff 1992). La première est une approche génétique qui cherche à définir l'évolution des fréquences alléliques et à déterminer les fréquences génotypiques à l'équilibre, en intégrant les mécanismes génétiques déterminant les traits phénotypiques étudiés et les contraintes génétiques. Cette approche est généralement mieux considérée que la suivante car elle s'intéresse à la transmission des différences génétiques au cours des générations, élément essentiel pour qu'une évolution soit possible (Roff 1992). Cependant, établir les mécanismes génétiques à l'origine d'un trait reste difficile et les interactions génotype*environnement compliquent la procédure.

La seconde approche, dite d'optimisation, a reçu une attention considérable dans la littérature et des critiques tout aussi nombreuses, essentiellement car elle ne repose pas sur une base génétique, et donc un potentiel évolutif (ex : Levins 1970,

Maynard-Smith 1978, Stearns & Schmid-Hempel 1987). Cette approche suppose que la sélection naturelle conduit les organismes à la combinaison optimale des traits pour laquelle la fitness est maximisée dans un environnement donné. Elle intègre les contraintes existantes, qui limitent les combinaisons de traits possibles, et suppose que la variation génétique est suffisante pour atteindre cet optimum.

La procédure de l'approche d'optimisation consiste à établir un ensemble de règles hypothétiques ou réelles, intégrant les contraintes écologiques, phylogénétiques et les trade-offs connus, qui décrivent l'histoire de vie de l'organisme étudié. Ces règles vont permettre de déterminer les combinaisons possibles, et de faire émerger celle(s) qui procure(nt) à l'organisme la fitness maximale dans un environnement donné. Cette stratégie optimale théorique peut ensuite être comparée à ce qui est réellement observé. Si la combinaison optimale théorique ne correspond pas à la combinaison observée, on ne supposera pas que la combinaison optimale n'a pas été sélectionnée (l'approche d'optimisation ne cherche pas à tester si la fitness est maximisée), mais que le modèle ayant permis d'établir les prédictions n'est pas adapté. En fonction des observations et résultats empiriques, de nouvelles règles d'histoires de vie, intégrant d'autres trade-offs ou d'autres contraintes écologiques, seront émises jusqu'à trouver le modèle qui colle le plus aux observations (Roff 1992).

Dans cette thèse, un raisonnement similaire à l'approche d'optimisation a été adopté. Nous avons cherché à déterminer dans quelle mesure les histoires de vie sélectionnées dans différents environnements permettent de maximiser la fitness des individus, en considérant un maximum de traits et de trade-offs connus et en intégrant des facteurs environnementaux alternatifs au climat.

b) Mesures de trade-offs

Reznick (1985) propose quatre approches pour mesurer des trade-offs.

La première est la mesure de corrélations phénotypiques. A l'échelle du phénotype, des corrélations entre traits sont établies, par exemple entre le nombre d'œufs pondus et la longévité d'une femelle. Cette approche est fortement critiquée car les trade-offs existants peuvent être masqués par des facteurs non pris en compte, comme la taille de l'individu. Elle ne prouve donc pas clairement qu'il y a un lien direct entre les traits considérés (Pease & Bull 1988, Roff 1992).

La seconde approche implique des manipulations expérimentales à l'issue desquelles on mesure l'effet du changement d'un trait étudié sur un ou plusieurs autres traits.

Afin d'établir un trade-off entre longévité et reproduction, on peut par exemple constituer deux lots d'individus, un qui aura la possibilité de se reproduire et l'autre non. La longévité sera ensuite comparée (ex : Ellers *et al.* 2000). La base fonctionnelle des trade-offs peut ainsi être mise en évidence.

La troisième méthode repose sur le calcul de corrélations génétiques entre traits, en comparant des individus de différents niveaux de parenté (Becker 1992), obtenus via divers designs de croisements.

Enfin, il est possible de calculer des corrélations génétiques entre traits via une sélection artificielle. Un trait est sélectionné artificiellement et l'on mesure la corrélation génétique avec d'autres traits. La critique majeure pour ce type d'expériences repose sur l'idée que la sélection naturelle n'agit pas sur un seul trait mais sur un grand nombre, contrairement à la sélection artificielle qui ne travaille que sur un trait (Roff 1992). La sélection artificielle d'un trait peut alors induire des conséquences sérieuses sur d'autres traits, qui seraient rapidement contre-sélectionnées dans la nature et pas en laboratoire. La sélection artificielle de changements positifs ou négatifs dans le comportement de phototaxie de *Drosophila melanogaster* induit par exemple une diminution de la vision des individus, qui serait certainement contre-sélectionnée en environnement naturel (Markow & Clark 1984).

Les deux premières méthodes ont été vivement critiquées entre autres car elles ne prouvent pas que les trade-offs ont une base génétique et qu'ils ont donc un potentiel évolutif (Reznick 1985, Roff 1992). Les manipulations expérimentales peuvent néanmoins apporter des informations intéressantes sur les trade-offs lorsque la question posée ne nécessite pas de montrer une base génétique. Les corrélations génétiques sont plus indiquées pour mettre en avant des trade-offs ayant un potentiel adaptatif, mais ces procédures sont lourdes et risquées car elles nécessitent de nombreuses lignées, un élevage sur de nombreuses générations qui peut être fragilisé par les effets délétères de la consanguinité, ce qui peut totalement masquer les corrélations que l'on cherche à mettre en évidence (Roff 1992).

Les mesures de trade-offs en laboratoire peuvent nécessiter certaines précautions expérimentales, la mise en évidence de coûts étant fortement liée aux conditions proposées aux organismes étudiés (Zera & Harshman 2001). Dans une expérience où les individus reçoivent une alimentation *ad libitum*, l'énergie absorbée peut par exemple compenser la dépense énergétique liée à un évènement reproducteur comme la ponte et masquer tout coût de la reproduction (Stearns 1992). Une limitation de la ressource alimentaire, certainement plus proche des conditions

rencontrées dans la nature, peut ainsi être nécessaire pour mettre en évidence ou accentuer un compromis entre traits (Zera & Harshman 2001).

2- Plasticité phénotypique des traits d'histoire de vie

A- Définition

Dès 1911, le botaniste Johannsen soulignait l'importance que l'environnement peut jouer dans l'interaction génotype-phénotype. Bien que Schmalhausen apporta en 1949 une première définition à ce « développement dépendant de l'environnement », c'est surtout celle de Bradshaw (1965) qui est la plus communément reprise : « Plasticity is shown by a genotype when its expression is able to be altered by environmental influences…it does not have any implication concerning the adaptive value of the change occurring, although many types of plasticity may have important adaptive effects ». La plasticité phénotypique peut donc être considérée comme la capacité d'un génotype à produire différents phénotypes lorsqu'il est exposé à différents environnements durant son ontogenèse (Pigliucci 2005). Elle constitue une réponse majeure à court-terme des organismes face aux variations environnementales. Les traits phénotypiques sont ainsi influencés par le génotype de l'organisme, l'environnement et l'interaction entre les deux.

B- Mise en évidence : normes de réaction

Cette plasticité peut être mise en évidence grâce à des mesures de normes de réaction, comme le fit Woltereck dès 1909 en mesurant l'effet de l'alimentation sur la taille et la forme de la carapace des daphnies. D'après de Jong (1990) « A reaction norm as coded for by a genotype is the systematic change in mean expression of a phenotypic character that occurs in response to a systematic change in an environmental variable. […] the reaction norm itself is a function, with the value of the environmental variable as argument and the genotypic value of the trait as function value; the phenotypic value in any environment would be determined by the genotypic value and some error." Concrètement, une norme de réaction pourra être déterminée en soumettant des organismes de génotype similaire (ex : clones) ou

proche à différentes valeurs du facteur environnemental que l'on souhaite tester, par exemple la température. On peut ainsi mesurer l'effet de la variation de ce facteur sur le trait étudié.

Les normes de réaction peuvent être linéaires, parfois du simple fait que seule une partie de la gamme environnementale a été testée, ou curvilignes. Dans ce cas, on observera une augmentation de la valeur du trait étudié avec le facteur environnemental testé jusqu'à atteindre un optimum (sommet de la courbe), au-delà duquel la valeur du trait décroit (Huey & Kingsolver 1989).

Les normes de réaction apportent diverses informations majeures quant à l'interaction génotype*environnement. Tout d'abord, l'élévation de cette norme de réaction indique la valeur du trait à travers la gamme environnementale. Considérons la norme de réaction du nombre d'œufs à l'émergence en fonction des ressources environnementales. Plus la norme de réaction sera élevée, plus les organismes auront d'œufs à l'émergence. Ensuite, une norme de réaction peut renseigner sur la gamme environnementale acceptable par l'organisme testé et sur la valeur optimale du facteur environnemental, pour laquelle le trait est maximisé. Le dernier élément est la pente de la courbe, qui traduit la force de la réponse du trait à la variable environnementale. Si cette pente augmente, l'environnement aura un effet d'autant plus fort sur le trait phénotypique, et inversement. Des travaux ont montré que cette dernière caractéristique pouvait présenter suffisamment de variation génétique entre individus pour être sélectionnée (Huey & Kingsolver 1989, Gilchrist 1995).

C- La plasticité, un avantage évolutif ?

Alors que Falconer (1952) et ses contemporains considéraient que la plasticité était un frein à la sélection naturelle et minimisaient son importance dans l'évolution des traits d'histoires de vie, des travaux plus récents ont mis en évidence l'existence d'une variation génétique et d'une héritabilité de la plasticité phénotypique dans la nature (Scheiner & Lyman 1989), et donc un potentiel adaptatif (Pigliucci 2005). Lyytinen *et al.* (2004) se sont par exemple intéressés à l'existence de deux formes saisonnières chez le papillon *Bicyclus anynana*, l'une présentant des ocelles durant la saison humide et l'autre sans, présente durant la saison sèche. Les résultats observés en exposant les deux formes à la prédation aviaire sur des feuilles vertes (saison humide) ou brunes (saison sèche) suggèrent que la forme sans ocelles serait sélectionnée en saison sèche car cryptique et que la forme à ocelles serait avantageuse

en saison sèche car intimidante. Ces deux régimes de sélection opposés auraient sélectionnés cette plasticité pour ce caractère. Une forte plasticité serait donc avantageuse dans des environnements fluctuants et pourrait théoriquement accélérer l'évolution grâce à une assimilation génétique en cas de persistance d'un changement environnemental (Waddington 1961). Toutefois, une assimilation génétique via la sélection naturelle n'a jamais été mise en évidence.

Certains coûts d'expression et de maintenance sont néanmoins associés à la plasticité phénotypique et expliquent en partie qu'une forte plasticité ne soit pas observée dans tous les environnements fluctuants (pour une synthèse, voir DeWitt *et al.* 1998). Ces coûts sont relatifs à la maintenance des mécanismes de régulation de la plasticité (León 1993), à la production d'un génotype plastique (Black & Dodson 1990), à l'acquisition de l'information qui permet de détecter les variations environnementales (Sih 1992), à l'instabilité développementale (Scheiner *et al.* 1991) ou à des répercutions génétiques comme par exemple des effets pléiotropiques délétères (Moran 1992). Tous ces coûts pourraient fortement contre-sélectionner la plasticité (van Tienderen 1997), surtout dans des environnements où les variations sont trop rapides, ou bien la détection des variations peu fiable (Callahan *et al.* 2008, Moran 1992). Cependant, les études s'intéressant à ce pan de la biologie évolutive sont encore trop rares pour dégager des conclusions claires, certaines montrant un effet significatif des coûts (Krebs & Feder 1998) et d'autres non (DeWitt *et al.* 1998, Relyea 2002).

D- Canalisation de la plasticité

Un phénotype constant peut être produit malgré des différences génétiques et des variations environnementales, on parle alors de canalisation (Waddington 1942). Diverses définitions de ce concept se recoupent comme la « similarité de l'expression de caractères phénotypiques sous différentes conditions de développement » (Zakharov 1992), la « suppression de variation phénotypique » (Wagner *et al.* 1997) ou « un processus par lequel la variation phénotypique est réduite » (Stearns *et al.* 1995). Cette canalisation peut être génétique (phénotype constant malgré la variation génétique) ou environnementale (phénotype constant malgré la variation environnementale). Une canalisation environnementale peut être mise en évidence en établissant la norme de réaction du trait étudié : plus la pente sera faible, plus l'on

considèrera que ce trait est canalisé (figure 3), d'après la définition de Stearns *et al.* (1995), reprise par d'autres auteurs (Liefting & Ellers 2008, Liefting *et al.* 2009).

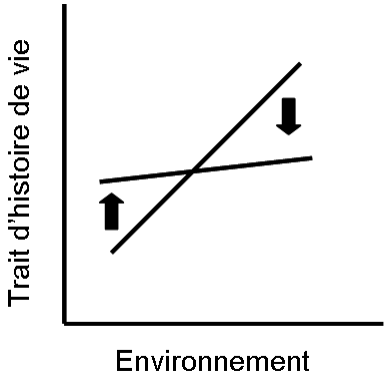

Figure 3. Exemple simple de canalisation environnementale. La pente de la norme de réaction est écrasée et un phénotype similaire est produit quel que soit l'environnement.

Il existe une opposition entre la capacité d'un trait à répondre rapidement et de façon optimale aux fluctuations environnementales (plasticité) et la nécessité de tamponner ce trait face à ces fluctuations (Nylin & Gothard 1998). Ainsi, des variations plastiques trop importantes de certains traits pourraient fortement réduire la fitness dans des conditions environnementales éloignées de l'optimum. La théorie prédit que les traits morphologiques et physiologiques devraient être plus plastiques dans les populations de milieux variables (Bradshaw 1965, de Jong 1995), alors que dans ces mêmes environnements, les traits les plus fortement liés à la fitness, comme la fécondité, devraient être plus constants. Ceux-ci seront ainsi tamponnés voire maximisés, quel que soit l'environnement (Richards *et al.* 2006, Stearns & Kawecki 1994, Wagner *et al.* 1997). Une corrélation positive entre variabilité environnementale et canalisation devrait donc être observée pour les traits fortement corrélés à la fitness. Des exemples de canalisation sont cités dans la partie suivante, section C.

3- Traits d'histoire de vie et climat

S'il est admis que tous les facteurs climatiques (précipitations, vent, photopériode…) peuvent affecter les traits d'histoire de vie, la température reste de très loin le facteur le plus étudié, surtout chez les ectothermes dont la température interne varie avec la température extérieure. Outre les comportements d'évitement dans l'espace ou dans le temps comme la migration ou la diapause, on distingue deux grands types de réponses au climat: une plasticité phénotypique thermo-dépendante, réponse à court-terme, et une sélection d'histoires de vie adaptées, réponse à long-terme. Cette dernière a été mise en évidence expérimentalement via des travaux de « sélection naturelle en laboratoire » ou grâce aux études d'adaptations locales. La plasticité elle-même peut être soumise à la sélection, ceci ayant été prouvé dans ces mêmes travaux de sélection et d'adaptations locales. Ces différents aspects sont abordés dans les paragraphes suivants.

A- Plasticité des traits

Une littérature très fournie appuie l'idée que de nombreux traits d'histoire de vie varient avec la température extérieure, surtout chez les ectothermes mais également chez les oiseaux et mammifères. Nous ne dresserons pas ici une liste exhaustive des traits affectés mais simplement ceux qui seront étudiés dans ce travail.

La température peut tout d'abord conditionner certains caractères comme le taux de métabolisme basal ou la taille. Le métabolisme joue un rôle central dans l'acquisition et l'allocation des ressources. Il s'agit d'un processus biologique qui repose sur des lois physiques et chimiques strictes, comme les principes de thermodynamique ou de réactions cinétiques. Il en résulte que le taux de métabolisme basal (c'est-à-dire au repos, sans aucune activité) est affecté par deux facteurs principaux : la taille de l'organisme et la température (Brown *et al.* 2004, Clarke 1993, Gillooly *et al.* 2001). Une augmentation du taux de métabolisme standard avec la température a été universellement observée chez les ectothermes vertébrés et invertébrés (ex : Chown 1997, Gatten *et al.* 1992, Zari 1991). Ainsi, plus la température est élevée, plus les ressources sont acquises et allouées rapidement entre les différentes fonctions comme la reproduction ou la survie. Un métabolisme basal élevé induit notamment un taux de croissance plus rapide et une activité locomotrice plus importante (Nylin & Gotthard 1998). En retour, une activité accrue, comme le

vol chez les insectes (Nation 2008), augmentera considérablement le taux de métabolisme actif (par opposition au métabolisme basal) et de ce fait l'énergie consommée.

Au contraire, la taille des organismes est généralement inversement corrélée à la température. Les individus se développant à basses températures sont généralement plus grands que ceux se développant à des températures supérieures, en raison d'un développement plus lent (Atkinson & Sibly 1997, Angilletta *et al.* 2004, Nylin & Gotthard 1998). On parle de « temperature size rule ». Si cette loi a été observée chez de nombreux ectothermes, on trouve cependant divers contre-exemples, notamment chez les insectes (Atkinson 1994). Une augmentation de la taille à basse température va généralement se traduire par une augmentation de la fécondité et/ou de la longévité, ces traits étant fortement taille-dépendants. La température de développement peut donc affecter ces traits via une modification de la taille. Afin de comparer des différences dans l'investissement des ressources entre les différentes fonctions à différentes températures, il est donc important de systématiquement corriger les traits par la taille.

Outre ces deux lois physiques qui vont pouvoir affecter l'ensemble des traits d'histoire de vie et qui sont parfois considérées comme des réponses purement physiologiques et non comme des exemples de plasticité phénotypique (van der Have & de Jong 1996), il a été établi qu'une diminution de la température peut induire une augmentation de la longévité ainsi que de la quantité de lipides (Colinet *et al.* 2007). Ces augmentations sont vraisemblablement la conséquence d'une simple diminution du taux de métabolisme, le rythme d'allocation des ressources étant alors ralenti. Une augmentation de la taille des œufs (Blanckenhorn 2000, Fischer *et al.* 2003) et une diminution du nombre d'œufs (Ernsting & Isaaks 2000, Stillwell & Fox 2005) ont également été observées avec une diminution de la température. Ces variations du volume et du nombre des œufs en réponse à la température sont considérées comme adaptatives, une variation génétique héritable de cette plasticité ayant été mise en évidence (Steigenga *et al.* 2005). Chez les insectes, la taille des œufs est positivement corrélée à la survie des jeunes. Produire de gros œufs constitue donc une stratégie permettant d'accroître la probabilité de survie des descendants dans des conditions stressantes comme des températures basses (Fischer *et al.* 2003). Cette augmentation de la survie des jeunes compenserait leur nombre réduit. En règle générale, les variations thermo-dépendantes du nombre d'œufs ne sont pas linéaires mais curvilignes. On observe une augmentation jusqu'à une température optimale

intermédiaire pour laquelle le trait est maximisé, puis une diminution (ex : Carrière & Boivin 2001, Eliopoulos & Stathas 2005). De même, les performances locomotrices des vertébrés ectothermes (Bennett 1980, Whitehead *et al.* 1989) ou le succès parasitaire des insectes parasitoïdes présentent souvent des normes de réaction curvilignes (Flinn 1998, Mbata *et al.* 2005).

B- Sélection naturelle en laboratoire

Bien que cette approche soit rare, d'après le peu de littérature existante, la mise en place de « sélection naturelle en laboratoire » est une approche particulièrement intéressante pour comprendre l'évolution des traits d'histoire de vie et des trade-offs. Elle consiste à élever des lignées à différentes températures durant des années et à inspecter les traits au fil des générations (Rose *et al.* 1990). Ces travaux, réalisés presque exclusivement sur des drosophiles, permettent de concrètement mettre en évidence l'évolution des traits d'histoire de vie en réponse à la température. Néanmoins, ceux-ci sont très coûteux en temps et les effets liés à la consanguinité peuvent être très forts.

En quatre ans de sélection, Huey *et al.* (1991) ont observé une évolution de la sensibilité thermique de la durée de développement de drosophiles. Les lignées élevées à basse température se développent plus vite au froid que celles élevées à haute température, la réciproque étant également mise en évidence. La résistance à la chaleur des lignées élevées à la température la plus élevée a également augmenté durant les quatre ans de sélection. Les mesures d'évolution de la longévité et de la fécondité réalisées par Partridge *et al.* (1995) ont par ailleurs montré que ces deux traits sont maximisés aux températures d'élevage, les drosophiles élevées à haute température vivant plus longtemps et produisant plus d'œufs à ces températures que les lignées élevées au froid, et réciproquement pour les lignées élevées au froid. On observe donc un décalage des normes de réaction dans le sens des températures d'élevage. La norme de réaction de la vitesse de marche de ces insectes évolue aussi dans le sens de la température d'élevage (Gilchrist *et al.* 1997). De plus, la taille du corps et de l'aile des drosophiles augmentent au cours des générations pour les lignées élevées aux basses températures (Anderson 1973, Lints & Bourgois 1987).

Il est intéressant de citer la seule étude existante, à notre connaissance, sur l'évolution de traits en réponse à l'humidité. Kennington *et al.* (2003) ont observé chez

Drosophila melanogaster une augmentation de la surface des ailes et de la taille corporelle chez des lignées élevées avec un faible taux d'humidité. Les individus sélectionnés seraient les plus gros, leur surface corporelle proportionnellement plus faible limitant les pertes d'eau.

C- Variations géographiques

Une des approches les plus courantes pour comprendre l'évolution des histoires de vie en réponse au climat consiste à échantillonner des populations d'une même espèce le long d'un gradient climatique (latitudinal ou altitudinal), à élever ces populations dans un environnement commun puis à comparer leurs traits. On parle d'études clinales. Celles-ci permettent de mettre en évidence des adaptations locales au climat, déterminées génétiquement. Bien que de nombreux facteurs climatiques (précipitations, vent, photopériode…) varient avec la latitude ou l'altitude, seule la température a été réellement considérée comme facteur de sélection. Si les drosophiles ont été particulièrement étudiées, les autres insectes n'ont reçu que peu d'attention dans ces études. L'essentiel des travaux présentés dans cette thèse repose sur ce type d'étude. Nous avons privilégié cette approche aux travaux de sélection en raison de leurs difficultés, évoquées précédemment.

a) Adaptations locales au climat

Des variations géographiques d'histoires de vie ont été montrées pour de nombreux traits. Ainsi, les populations de drosophiles des milieux les plus chauds sont plus résistantes à un stress thermique chaud, alors que les populations de milieu froid sont plus résistantes à un stress thermique froid (Collinge *et al.* 2006, Hoffmann *et al.* 2002 ; Sørensen *et al.* 2005), un trade-off étant parfois suggéré entre ces deux capacités de résistance. Un pattern similaire est observé par les mêmes auteurs pour la résistance à la dessiccation, plus élevée dans les régions chaudes. Il s'agit à notre connaissance du seul trait, avec la taille, ayant été associé à des variations géographiques d'humidité et non de température dans des études clinales. La résistance au jeûne des drosophiles ne semble en revanche pas varier selon un gradient climatique (Gilchrist *et al.* 2008).

Des évidences pour des clines latitudinaux existent également pour la taille, qui augmente avec la latitude (ex : James *et al.* 1997), ou encore la charge alaire, qui est

négativement corrélée à la latitude chez les populations européennes de *D. melanogaster* (Gilchrist & Huey 2004).

Schmidt *et al.* (2005) ont apporté une des études les plus complètes sur les stratégies de reproduction de *Drosophila melanogaster* en comparant des populations nord-américaines échantillonnées le long de la côte Est. Ils ont observé que les femelles du sud des Etats-Unis vivaient moins longtemps et se reproduisaient plus tôt alors que les populations du nord vivaient plus longtemps émergeaient avec plus de lipides, et se reproduisaient plus tard. La faible longévité observée dans les milieux les plus chauds (Sud) induit la sélection des individus se reproduisant plus tôt, au détriment des réserves lipidiques. Ces combinaisons opposées de traits, observées également par Mitrovski & Hoffmann (2001) ou Schmidt & Paaby (2008), seraient donc sélectionnées en réponse au climat. Cependant, d'après l'étude de Schmidt & Paaby (2008), il semblerait qu'une part de cette variance soit expliquée par la fréquence élevée des « génotypes diapausants» dans les populations du Nord, bien que les conditions climatiques d'origine jouent également un rôle important.

Des adaptations locales du comportement ont également pu être mises en évidence. Le long d'un gradient altitudinal en Argentine, Dahlgaard *et al.* (2001) ont observé une variation dans les horaires d'activité de drosophiles, les femelles d'altitude élevées étant plus actives l'après-midi et celles de faible altitude au crépuscule. L'activité accrue de ces dernières en fin de journée, et notamment l'oviposition, aurait été sélectionnée pour limiter les expositions aux températures élevées rencontrées durant la journée à basse altitude.

b) Variations de la plasticité phénotypique

Les études clinales ont également permis de mettre en évidence des variations géographiques des normes de réaction et du niveau de la plasticité phénotypique pour divers traits, bien que cette question ait été peu étudiée (Figure 4).

Le premier élément qui puisse varier est l'optimum et la gamme de températures dans laquelle l'individu reste performant. On peut ainsi observer un décalage des normes de réaction vers les températures chaudes pour les populations de milieu chaud, et inversement, comme observé dans les travaux de sélection naturelle en laboratoire (voir ci-dessus). Trotta *et al.* (2006) ont par exemple noté que la norme de réaction du nombre de descendants produits par *D. melanogaster* est décalée vers les températures élevées pour les populations des zones les plus chaudes,

et inversement pour les populations des régions froides. Liefting *et al.* (2009) et Morin *et al.* (1999) ont respectivement observé un résultat similaire pour la durée de développement de *D. serrata*, et la longueur du thorax et de l'aile chez *D. melanogaster* et *D. simulans*.

Les variations du degré de plasticité ont également été étudiées. Si certaines études ont associé le degré de plasticité à la température moyenne d'origine, comme une plasticité de la durée de développement plus importante pour des populations de milieux chauds (Huey *et al.* 1991, James & Partridge 1995), la majorité des auteurs se sont intéressés à l'impact de la variabilité thermique sur le degré de plasticité. En comparant des populations de collemboles élevées à différentes températures, Liefting & Ellers (2008) ont effectivement observé que le taux de croissance, un trait lié à la fitness, des individus de forêts (milieu thermiquement stable) était plus plastique que celui des individus issus de landes (milieu plus variable). De même, Liefting *et al.* (2009) ont observé que le taux de développement de *Drosophila serrata* issues de climat tempéré (milieu variable) était plus canalisé que les populations de climat tropical, alors que les traits morphologiques étaient plus plastiques dans ces populations de climat tempéré. A l'inverse, James *et al.* (1997) n'ont trouvé aucune variation latitudinale dans la plasticité de la durée de développement ou de traits morphologiques chez *D. melanogaster*.

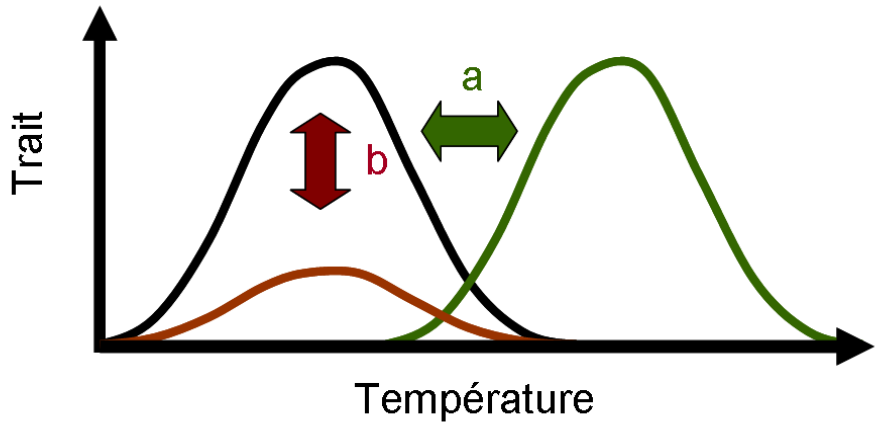

Figure 4. Les travaux de sélection naturelle en laboratoire et les études clinales ont mis en évidence deux changements possibles des normes de réaction en réponse au climat: (a) un décalage de cette norme aux températures expérimentées (b) une augmentation ou une diminution de la plasticité en fonction de la variabilité environnementale.

Ces variations géographiques de normes de réaction, déterminées génétiquement, peuvent renforcer ou s'opposer aux effets environnementaux, on parle alors de « cogradient » ou de « countergradient variation », qui seront développées dans le paragraphe suivant.

c) « Countergradient variation » *vs* « cogradient variation »

Décrits en premier lieu par Levins (1968) puis repris plus tard par Conover & Schultz (1995), les termes de « countergradient variation » (CnGV) et « cogradient variation » (CoGV) renvoient à des distributions géographiques particulières de génotypes, à l'égard de l'environnement. Ces patterns ont été étudiés afin de comprendre la variabilité phénotypique géographique rencontrée dans la nature et dans quelle mesure celle-ci est influencée par des facteurs génotypiques ou environnementaux.

La variance phénotypique d'un caractère quantitatif peut être décomposée comme suit :

$V_P = V_G + V_E + V_{G*E} + 2\,Cov(G,E)$

ou V_G = part de variance résultant d'effets génétiques,

V_E = part de variance résultant d'effets environnementaux

V_{G*E} = interaction entre génotype et environnement

Cov(G,E) = covariance entre les sources de variance génotypique et environnemental. Ce terme désigne le degré auquel des génotypes ayant un effet sur l'expression phénotypique de traits ne sont pas distribués de façon hasardeuse dans des environnements qui font varier ces mêmes traits.

Cette covariance peut augmenter ou diminuer la variance phénotypique, selon que les effets génotypiques et environnementaux sur le phénotype se renforcent (covariance positive) ou s'opposent (covariance négative) le long d'un gradient. Elle est positive dans un gradient où des individus avec une caractéristique héritable dans un caractère phénotypique sont trouvés dans des environnements qui modifient le caractère dans la même direction. L'exemple le plus répandu est l'existence de plantes avec un génotype « petite taille » à haute altitude, dans un environnement qui favorise la petite taille (Clausen *et al.* 1940). Ce type de gradient correspond à une « cogradient variation ». Les travaux cités en partie « Adaptations locales au climat » mettent ainsi

en évidence des « cogradient variations » pour la fécondité, la longévité et la teneur en lipides chez les drosophiles (Schmidt *et al.* 2005, Mitrovski & Hoffmann 2001). De même, les traits morphologiques suivent généralement une « cogradient variation », comme par exemple la taille des œufs (Azevedo *et al.* 1996) ou la taille de l'organisme (James *et al.* 1997), bien que des contre-exemples existent pour ce trait (Levins 1969). La covariance est négative lorsque les génotypes sont distribués de telle façon que les effets génétiques et environnementaux sur le caractère phénotypique s'opposent le long du gradient étudié. On parle alors de « countergradient variation » (CnGv), qui devrait résulter en une réduction de la variance phénotypique le long du gradient étudié.

Levins (1969) fut le premier à décrire un exemple de CnGv. Il échantillonna à Puerto Rico des drosophiles le long d'un gradient altitudinal, et donc thermique. Il éleva ces insectes à une même température et constata que les mouches de basse altitude étaient plus grosses alors qu'elles provenaient d'un milieu plus chaud, où une petite taille devrait être observée. Dans ce cas, le gradient thermique s'oppose à un gradient d'humidité : les zones de basse altitude sont les plus sèches. L'humidité n'ayant pas d'effet direct sur la taille des drosophiles durant le développement, une grande taille dans les zones les plus sèches aurait été sélectionnée pour limiter les risques de dessiccation. Le génotype « grande taille » sélectionné à basse altitude en réponse à l'humidité irait donc à contre-sens de la réduction de taille induite par les températures élevées. Levins décrivait ainsi cette « countergradient variation » comme une opposition entre effet de la sélection et de l'influence environnementale durant le développement.

Plusieurs travaux sur le taux de croissance ont également mis en avant une CnGv pour ce trait. En élevant des grenouilles *Rana sylvatica* provenant de climats différents, Ligon & Skelly (2009) ont observé que les individus des zones les plus froides présentent un taux de croissance plus élevé que ceux des milieux les plus chauds, lorsqu'ils sont élevés à même température. Ces résultats sont à relier à l'hypothèse de « Metabolic Cold Adaptation », qui sera développée dans le prochain paragraphe. Outre ces patterns géographiques fréquents pour les traits physiologiques que sont le taux de croissance et le taux de métabolisme (Conover *et al.* 2009), les cas de CnGv restent exceptionnels et doivent être considérés au cas par cas.

Deux méthodes principales de mises en évidence de CoGv ou CnGv existent : (1) échantillonner des populations le long d'un gradient environnemental et mesurer en laboratoire les normes de réaction en réponse au facteur principal variant le long de

ce gradient (ex : température pour un gradient altitudinal ou latitudinal) ou (2) transplanter réciproquement des populations d'un environnement donné vers un environnement contrasté. Pour les cas les plus simples, si l'effet du génotype (i.e. origine de l'organisme) va dans la même direction que l'effet de la variable environnementale sur le phénotype, on supposera une CoGv. Si les deux s'opposent, on supposera une CnGv. Les cas plus complexes apparaissent lorsque l'interaction entre le génotype et l'environnement est forte, résultant en des pentes de normes de réaction opposées entre les différentes populations.

d) Un pattern géographique central : la Metabolic Cold Adaptation

Si l'on sait que le taux de métabolisme basal augmente de manière universelle avec la température chez les ectothermes, dans la limite de températures ordinairement observées dans la majorité des écosystèmes (0 à 40°C), l'évolution de ce trait en réponse au climat est encore sujette à débat. L'hypothèse la plus répandue est la « Metabolic Cold Adaptation (MCA) hypothesis » (Wohlschlag 1960, Clarke 1993), considérée comme un exemple fort de « countergradient variation » (Conover & Schultz 1995). Elle a été développée lorsque des chercheurs ont observé que dans la nature, les espèces/populations de milieu polaire présentaient une activité similaire à celles de milieux plus chauds, alors que le froid devrait ralentir l'activité des organismes en milieu polaire. En élevant ces espèces/populations de climats contrastés à une température commune, on observe que les individus des zones les plus froides ont un taux de métabolisme et un taux de croissance plus élevés que ceux des zones chaudes (ex : Álvarez *et al.* 2006, Cano & Nicieza 2006, Berrigan & Partridge 1997). Par la suite, d'autres travaux de laboratoire ont permis de mettre en évidence que des individus se développant à basse température présentent à l'âge adulte, et pour une même température, un taux métabolique plus élevé que ceux se développant à haute température (Berrigan 1997). Ce phénomène de « compensation thermique » (autre nom donné à la MCA hypothesis) a également été observé avec les fluctuations thermiques saisonnières.

Cette compensation thermique permettrait de maintenir les activités physiologiques et une activité locomotrice suffisantes dans des zones ou durant des périodes froides,

notamment via une production de mitochondries supérieure à celle des zones chaudes (Hochachka & Somero 2002).

e) Les facteurs confondants

De nombreux autres facteurs environnementaux, abiotiques ou biotiques, varient avec le climat le long de gradients latitudinaux ou altitudinaux et peuvent avoir un effet confondant ou un effet opposé à ce climat. Le plus étudié des facteurs abiotiques reste probablement la photopériode, rarement dissociée du climat. Plusieurs auteurs ont ainsi mis en évidence des variations géographiques de sensibilité à la photopériode induisant une entrée en diapause, avec notamment une sensibilité moindre à la photopériode pour les populations de faible latitude (ex : Bradshaw *et al.* 2004, Ito & Nakata 2000). La diapause jouant un rôle important dans les adaptations locales de fécondité et de longévité (Schmidt & Paaby 2008), on peut supposer que la photopériode, en plus du climat, joue un rôle dans les adaptations locales de ces traits.

Le climat peut également être à l'origine de modifications des conditions biotiques dans le milieu, comme la composition spécifique des communautés (au sens strict) dans lesquelles les organismes évoluent. Moore *et al.* (2002) suggèrent ainsi que l'abondance élevée des parasites aux latitudes tropicales, en raison de conditions climatiques favorables (Moller 1998), expliquerait le faible taux de testostérone observé chez les oiseaux vivant à ces latitudes, en raison de son effet immunosuppresseur. Kraaijeveld & Godfray (1999) ont quant à eux constaté que des variations d'espèces d'hôtes rencontrées le long d'un gradient climatique pouvaient expliquer les variations latitudinales de virulence du parasitoïde de drosophiles *Asobara tabida.* En effet, au Sud de l'Europe, une seule espèce d'hôte, *Drosophila melanogaster*, est présente, alors qu'au Nord cette espèce est sympatrique d'une espèce de milieu tempéré, *D. subobscura.* Contrairement à la première, cette espèce moins résistante au parasitisme est incapable d'encapsulation, une réponse immunitaire empêchant le développement de l'œuf de parasitoïde (Russo *et al.* 1996). Une virulence accrue aurait donc été sélectionnée au Sud, les parasitoïdes devant nécessairement parasiter un hôte résistant. De plus, le réchauffement global actuel modifie localement la composition des communautés, les espèces de basse latitude pouvant s'étendre vers les hautes latitudes. Dans l'hémisphère Nord, les espèces nordiques se retrouvent confrontées à de nouvelles espèces compétitrices venues du Sud. C'est par exemple le cas des parasitoïdes *Leptopilina heterotoma* et *Asobara*

tabida présentes dans la vallée Rhodanienne qui se retrouvent dorénavant en compétition avec *L. boulardi*, un parasitoïde méditerranéen remontant vers le Nord. Ce nouveau compétiteur aurait induit une modification des traits d'histoire de vie des espèces autochtones (Fleury *et al.* 2004).

La sélection d'histoires de vies différentes le long d'un gradient climatique pour un organisme peut être également attribuée à son interaction avec d'autres espèces dont les traits varient avec le climat. Malo & Baonza (2002) ont par exemple observé une augmentation de la taille des pollinisateurs ainsi que des fleurs le long d'un gradient altitudinal en Espagne. Dans cette étude, la variation géographique de la taille des fleurs a été attribuée à la contre-sélection des petites fleurs à haute altitude qui ne peuvent être pollinisées par les insectes de grande taille présents à cette latitude. Dans leurs travaux sur les variations géographiques du nombre d'ovarioles de *Drosophila hibisci*, Starmer *et al.* (1997) ont tenté de discriminer la réponse liée au climat de celle liée aux traits morphologiques des hibiscus, plante dont dépendent étroitement les drosophiles pour leur alimentation et leur reproduction. Les auteurs n'ont trouvé qu'un faible effet du climat et aucun effet des traits des hibiscus utilisés par les drosophiles.

Enfin, les variations géographiques de distribution des ressources et de caractéristiques de l'habitat associées au climat peuvent également être à l'origine de variations géographiques d'histoire de vie. Dans leur étude citée précédemment sur *Drosophila hibisci*, Starmer *et al.* (1997), avaient également inclus des caractéristiques physiques des habitats comme la densité en arbres, sans toutefois montrer un quelconque effet de ces facteurs. A l'inverse, en comparant des papillons mâles de milieux agricoles ouverts et fermés, Merckx *et al.* (2008) ont observé une pilosité thoracique plus importante chez les individus de milieu ouvert. Ce trait aurait été sélectionné dans cet habitat pour limiter les déperditions de chaleur liées aux flux d'airs, plus importants en milieux ouverts que fermés, et augmenterait ainsi les performances locomotrices des individus. Ellers & van Alphen (1997) ont quant à eux échantillonné des femelles *Asobara tabida* dans le nord et le sud de l'Europe, qu'ils ont élevées dans des conditions similaires. En leur présentant différentes quantités d'hôtes, ils ont clairement mis en évidence un effet de l'habitat d'origine des femelles sur l'allocation des ressources entre différents traits. Les femelles du nord émergent avec plus de lipides de réserves et moins d'œufs que celles du sud, mais ces deux traits sont plus plastiques chez les premières. Ces réponses peuvent s'expliquer par des caractéristiques différentes des habitats d'origine, qui peuvent être attribuées au

climat. Dans le nord, les flux de sève représentent la majorité des sites de ponte des drosophiles, et donc des parasitoïdes, alors que dans le sud, ce sont les fruits en fermentation des vergers qui constituent les principaux sites de ponte. Cette opposition entre les sites du Nord, très espacés avec des distances de vol inter-patch très variables, et ceux du Sud, rapprochés et plus uniformes, aurait conduit à la sélection des adaptations locales observées. Un haut degré de plasticité aurait été sélectionné dans les milieux hétérogènes du Nord alors que la richesse et l'homogénéité des habitats du Sud, auraient favorisé un investissement maximal dans la reproduction. Toutefois, bien que ces variations aient été attribuées aux différences de distribution d'hôtes, les variations climatiques comme facteurs explicatifs ne peuvent être rejetées car les deux facteurs varient ensemble.

Malgré l'importance des covariations du climat avec d'autres facteurs abiotiques et biotiques, ces derniers ne sont que très rarement considérés dans les études clinales. A notre connaissance aucun auteur, hormis Starmer *et al.* (1997), n'a entrepris de différencier les effets directs du climat de ceux des facteurs associés, certainement en raison de la complexité d'une telle étude. La méthode proposée par ces derniers auteurs pour discriminer l'impact du climat et de l'habitat sur l'évolution des histoires de vie consistait à réaliser un échantillonnage sur de nombreux sites en quantifiant systématiquement le maximum de variables environnementales, biotiques et abiotiques, pour établir des corrélations entre ces dernières et les traits d'histoires de vie. Néanmoins, quantifier précisément toutes les variables environnementales, dont la distribution des ressources, sur un grand nombre de sites reste extrêmement fastidieux. Une méthode alternative consisterait à comparer pour différents climats des sites dont seules les variables biotiques testées varient. Néanmoins, ceci reste extrêmement compliqué puisque le climat influence directement ces mêmes variables environnementales.

4- Un modèle privilégié : les parasitoïdes

Les parasitoïdes sont des organismes dont la vie adulte est généralement libre mais dont la vie larvaire est parasitaire. Les femelles adultes vont activement rechercher un hôte pour y déposer un ou plusieurs œufs à l'intérieur (endoparasite) ou à l'extérieur (ectoparasite) du corps de celui-ci. Les stades immatures tirent ensuite leur nourriture du corps de leur hôte, induisant leur mort en résultat direct ou indirect de leur développement (Eggleton & Gaston 1990). On dénombre un total de 87 000 espèces dans le monde, dont 65 000 espèces d'Hyménoptères (Quicke 1997).

Les parasitoïdes représentent un modèle privilégié en biologie évolutive, et ce pour deux raisons essentielles. Tout d'abord, le nombre de descendants, et donc la fitness, est directement dépendant du nombre d'hôtes parasités (Wajnberg *et al.* 2008). On suppose donc une pression de sélection extrêmement forte sur la fécondité et le timing de la reproduction. Ensuite, une majorité de parasitoïdes n'est pas capable de synthétiser des lipides durant la vie adulte alors que ceux-ci sont impliqués dans les grandes fonctions biologiques. Ces insectes doivent composer avec les lipides acquis durant le stade larvaire (Visser & Ellers 2008, Visser *et al.* 2010). Cette contrainte énergétique induit que ce modèle biologique est particulièrement intéressant pour comprendre les pressions sélectives liées à l'allocation de ressources entre les différents traits d'histoire de vie et les trade-offs physiologiques.

A- Stratégies de reproduction des parasitoïdes et trade-offs

Roff (1992) considère que la première décision d'un organisme est de savoir quand se reproduire. Chez les parasitoïdes, cette phrase prend tout son sens dans la mesure où les ressources limitées issues de l'hôte seront investies dès le développement larvaire, soit dans la reproduction initiale soit dans le soma et les réserves énergétiques disponibles pour la vie adulte.

Les parasitoïdes sont soumis à deux contraintes essentielles dans la détermination de l'investissement optimal dans la reproduction : les risques de limitation en œufs (egg-limitation) et de limitation en temps (time-limitation). On parle de limitation en œufs lorsque le nombre d'hôtes rencontrés excède le nombre d'œufs matures dans les

ovaires ; alors que la limitation en temps s'applique à des parasitoïdes dont le nombre d'œufs matures excèderait le nombre d'hôtes rencontrés. Le nombre potentiel d'hôtes rencontrés devrait donc fortement déterminer l'allocation optimale des ressources entre fécondité (qui permet d'éviter une limitation en œufs) et longévité (qui permet d'éviter une limitation en temps). Diverses études de modélisation ou de terrain (comptage de nombre d'œufs d'individus échantillonnés dans la nature) ont révélé des résultats contradictoires, indiquant qu'une limitation en temps (Driessen & Hemerik 1992, Donaldson & Walter 1991, Weisser *et al.* 1997), comme une limitation en œufs (Casas *et al.* 2000, Heimpel & Rosenheim. 1998) peuvent être observées, souvent dans des proportions variables au sein d'une même population. La stratégie de maturation des œufs conditionne fortement les risques de limitation en œufs ou en temps.

On distingue chez les parasitoïdes deux grands types de stratégies de maturation des œufs: la proovogénie et la synovogénie (Flanders 1950). Elles se différencient par le rapport entre fécondité à l'émergence et fécondité potentielle totale, un continuum existant entre ces deux stratégies. Jervis *et al.* (2001) ont proposé l'utilisation de ce rapport, baptisé indice d'ovogénie (OI), pour comparer les stratégies de maturation d'œufs. Cet indice va de 0 à 1, les femelles émergeant avec la totalité de leurs œufs matures ayant un indice de 1.

Les **espèces proovogéniques** se caractérisent par un investissement maximal dans la fécondité initiale, puisque les femelles émergent avec la totalité des œufs matures et ne produisent pas d'œufs additionnels durant la vie adulte (OI = 1). Ainsi, si ces espèces ne sont pas limitées par le nombre d'œufs à l'émergence, elles ne peuvent ajuster leur investissement dans la reproduction en fonction du nombre d'hôtes rencontrés. Elles seront donc fortement contraintes dans un environnement hétérogène (Ellers *et al.* 2000). Leur longévité est inférieure à celle des espèces synovigéniques, et il est probable que peu de ressources soient allouées au corps gras (Jervis *et al.* 2001), et donc à la maintenance de l'individu et au vol. Les modèles développés par Ellers & Jervis (2003, 2004) ont montré que trois facteurs essentiels peuvent augmenter la probabilité qu'une stricte proovogénie soit une stratégie optimale : une petite taille corporelle, qui limite les déplacements possibles entre patchs, des œufs de grande taille, et une distribution uniforme des patchs d'hôtes.

Une stricte proovogénie est minoritaire chez les parasitoïdes puisqu'elle n'est détectée que chez 1,8% des parasitoïdes parmi 638 espèces étudiées (Jervis *et al.* 2001). Elle

concerne essentiellement deux familles, les Mymaridae et les Eucoilidae (dont fait partie *Leptopilina boulardi*). Ces espèces possèdent généralement les plus larges spectres d'hôtes potentiels (Jervis *et al.* 2005).

Les **espèces synovigéniques** se caractérisent quant à elles par un investissement différé dans la reproduction, les femelles étant capables de produire des œufs additionnels durant la vie adulte (0I < 1). Elles peuvent être caractérisées comme plus ou moins proovogéniques, en fonction de la proportion entre fécondité à l'émergence et fécondité potentielle totale (Quicke 1997). Plus ce rapport sera élevé, plus les espèces tendront vers la proovogénie. Leur investissement dans la reproduction et la longévité est donc flexible et peut être ajusté au nombre d'hôtes rencontrés. Une limitation en œufs apparaitra chez ces espèces lorsque le taux de maturation des œufs est inférieur au taux de rencontre des hôtes, comme observé chez *Encarsia pergandiella* (Hunter 1993) ou *Sympiesis sericeicornis* (Casas *et al.* 1993). Ces risques de limitation en œufs sont d'autant plus importants en début de vie.

Les espèces considérées comme synovigéniques représentent plus de 98% des 638 espèces testées par Jervis *et al.* (2001), avec de fortes variations dans les valeurs d'indice d'ovogénie, de 0 (surtout chez les Ichneumonidae et Bethylidae) à 0.8. Les espèces pouvant se nourrir sur leurs hôtes à l'état adulte ainsi que les espèces capables de résorber leurs œufs ne se retrouvent que dans cette catégorie (Jervis *et al.* 2001). Une telle stratégie serait sélectionnée dans des environnements hétérogènes où une flexibilité de l'investissement dans la reproduction et/ou la longévité est nécessaire et où le succès reproducteur des femelles est essentiellement lié à leurs capacités de dispersion (Ellers *et al.* 1998).

Il existe chez les synovogéniques une corrélation négative entre longévité et proportion d'œufs matures à l'émergence, observée à l'échelle inter- et intraspécifique (Jervis *et al.* 2001, Jervis & Ferns 2004). En comparant différentes espèces d'Hyménoptères, Jervis & Ferns (2004) ont observé que celles qui tendent le plus vers la proovogénie vivent le moins longtemps. De même, Jervis *et al.* (2001) suggèrent que la quantité de ressources allouées au corps gras augmente avec le degré de synovogénie.

B- Ressources énergétiques chez les insectes et les parasitoïdes

La balance des nutriments est un point essentiel dans les histoires de vie des insectes (Nation 2008), puisque tous ne sont pas alloués aux mêmes organes et fonctions, et qu'ils peuvent être à la base de trade-offs physiologiques entre traits. On distingue trois classes principales de nutriments, stockés dans le corps gras et l'hémolymphe : les sucres (ou carbohydrates), les lipides et les protéines. Chez les parasitoïdes, les principaux sites d'allocation des ressources sont les ovaires (lipides et protéines surtout) et le corps gras (Ellers 1996, Ellers & van Alphen 1997, Rivero & Casas 1999, Olson *et al*. 2000).

Les sucres sont les premiers éléments mobilisés en cas de besoin, et leur conversion en énergie est la moins coûteuse (Nation 2008), notamment via le glucose qui constitue une molécule de base servant à produire de l'ATP par combinaison avec de l'oxygène. Les autres carbohydrates doivent être fractionnés en glucose et fructose pour pouvoir être utilisés pour la production d'énergie. Ils peuvent être stockés dans l'organisme sous forme de glycogène.

Chez les parasitoïdes, les sucres peuvent être obtenus par piqure nutritionnelle (Heimpel & Collier 1996) ou en s'alimentant de nectar, de fruits, de miellat ou d'exsudat de plantes (Jervis *et al.* 1993). Ces carbohydrates sont impliqués dans la maintenance et la longévité de l'organisme (Giron *et al.* 2004, Jervis & Kidd 1986), dans la production et la maturation d'œufs (England & Evans 1997, Olson & Andow 1998), dans les déplacements, et peuvent être mobilisés pour le vol sur de courtes distances (Suarez *et al.* 2005). Les sucres peuvent également jouer un rôle essentiel dans la résistance au froid chez les insectes (Sinclair *et al.* 2003).

Les lipides, essentiellement stockés dans le corps adipeux et dans une moindre mesure dans les muscles alaires, représentent une ressource énergétique majeure chez les parasitoïdes. Bien qu'ils soient plus difficiles à mobiliser que les sucres, l'énergie qu'ils génèrent est de loin supérieure (129 moles d'ATP/mole de lipide contre 36 moles d'ATP/mole de glucose). On considère que chez les parasitoïdes, les ressources énergétiques sont essentiellement stockées sous forme de lipides (Rivero & Casas 1999). Ces nutriments sont à la base de trade-offs dans l'allocation des ressources entre les différentes fonctions, notamment entre maintenance et reproduction (Ellers 1996, Ellers & van Alphen 1997). Ces contraintes physiologiques sont d'autant plus fortes chez les parasitoïdes que la majorité d'entre eux ne peut synthétiser de lipides (Visser *et al.* 2010). Leur rôle est donc fondamental dans la compréhension des histoires de vie. Ils peuvent être mobilisés pour accroître la longévité (Ellers 1996) et constituent une ressource essentielle pour la production d'œufs (Giron & Casas 2003,

Ellers & van Alphen 1997). Ils sont également impliqués dans les vols sur de longues distances, un mode de locomotion extrêmement couteux en comparaison de la marche et induisant une augmentation du taux de métabolisme de 50 à 100 fois supérieur au métabolisme de repos (Ellers *et al.* 1998, Nation 2008**)**. Les lipides interviennent aussi dans la diapause (Canavoso *et al.* 2001) et dans la résistance au froid et à la dessiccation chez les insectes (Worland *et al.* 2004).

On a longtemps considéré qu'aucune lipogenèse adulte n'existait chez les parasitoïdes, ceux-ci pouvant prélever les lipides nécessaires à la vie adulte dans les nutriments de l'hôte, durant le stade larvaire (Visser & Ellers 2008). Cependant, Visser *et al.* (2010) ont récemment décrit une synthèse de lipides chez 7 espèces parmi 24 testées. D'après ces auteurs, une capacité à synthétiser des lipides serait réapparue au cours de l'évolution chez certaines espèces généralistes, parfois éloignées phylogénétiquement. Les généralistes, qui parasitent une large gamme d'hôtes, seraient moins efficaces pour les manipuler et utiliser leurs lipides. La lipogenèse, permettant de pallier à la moindre quantité de lipides extraits de l'hôte, serait alors réapparue au cours de l'évolution.

Les protéines peuvent quant à elles servir différentes fonctions dont la production d'œufs (Boggs 1981, Giron & Casas 2003), la construction de tissus corporels (Nation 2008), la métamorphose et la diapause (Hahn & Denlinger 2007). La proline est également impliquée dans le vol sur longue distance, en proportion plus faible que les lipides (Nation 2008). De même que pour les lipides, il existerait des compromis dans l'allocation des protéines entre différentes fonctions (Zera & Zhao 2006).

5- Plan de thèse

Dans cette thèse, située à l'interface de l'écologie et de la biologie évolutive, deux questions principales ont été étudiées, en prenant pour modèles plusieurs espèces de parasitoïdes de drosophiles:

{1}

Quelles adaptations locales des traits d'histoire de vie ont été sélectionnées en réponse au climat et à un facteur biotique dépendant du climat, la présence d'espèces compétitrices?

{2}

Quels facteurs environnementaux et intrinsèques aux parasitoïdes peuvent modifier la plasticité phénotypique des traits d'histoire de vie ?

La première question a été développée dans les deux premiers articles présentés dans ce manuscrit. Je me suis tout d'abord intéressé à l'importance du climat dans la sélection des histoires de vie (premier article). Ensuite, dans le cadre du stage de Master 2 de Chloé Vayssade que j'ai co-encadré, nous nous sommes intéressés aux effets de la composition des communautés et de la compétition interspécifique, dépendantes du climat, sur l'évolution des traits d'histoire de vie (deuxième article).
La deuxième question a été abordée sous la forme de deux articles traitant de la plasticité des traits d'histoire de vie en réponse à la température chez des populations iraniennes de *L. boulardi*. Dans cette partie, nous nous sommes intéressés à l'évolution des normes de réaction et de la force de la plasticité phénotypique, en réponse au climat et aux caractéristiques intrinsèques des organismes, dont les stratégies de maturation des œufs. Nous nous sommes focalisés sur la plasticité du taux de métabolisme (troisième article) puis de la fécondité et de certains nutriments (quatrième article).

Local adaptations of life history traits of a *Drosophila* parasitoid, *Leptopilina boulardi*: does climate drive evolution?

Dans ce premier article, nous avons tenté de mettre en évidence des adaptations locales des traits d'histoire de vie du parasitoïde de drosophiles *Leptopilina boulardi* en réponse au climat, en comparant des populations iraniennes originaires de climats contrastés. Nous avons observé des différences d'histoires de vie très importantes, comme des oppositions proovogénie/synovogénie et capacité/incapacité de lipogenèse entre populations. Cependant, les résultats obtenus nous ont amenés à considérer que la distribution des hôtes dans l'habitat d'origine, dépendante du climat, aurait joué un rôle plus important que le climat lui-même dans la sélection de ces traits.

Life-history trade-offs' shift in response to community changes: the case of the Drosophila parasitoids from the Rhône Valley

Outre la distribution des hôtes, de nombreux autres facteurs biotiques varient avec le climat, comme la distribution des espèces. Dans la vallée du Rhône, l'aire de répartition de *L. boulardi* s'étend à l'heure actuelle vers le Nord, certainement en raison d'une hausse des températures. Les espèces de parasitoïdes autochtones de cette zone, *L. heterotoma* et *A. tabida*, se retrouvent dorénavant confrontées à un nouveau compétiteur supérieur. L'université de Lyon suit annuellement la remontée de *L. boulardi* depuis plusieurs années, les dates où ces espèces autochtones se retrouvent en compétition avec l'espèce envahissante sont donc connues. Ce système offre de plus l'avantage de présenter un climat similaire sur le front d'invasion de *L. boulardi*. En comparant des populations naturelles des deux parasitoïdes autochtones soumises depuis de nombreuses (Sud) ou quelques années (Nord) à *L. boulardi*, nous avons cherché à déterminer si cette compétition a pu modifier les histoires de vie des parasitoïdes autochtones. Nous avons testé en particulier l'hypothèse de Price (1974), qui prédit qu'une fécondité supérieure devrait été sélectionnée dans les environnements où la mortalité des descendants est forte, le sud dans notre cas.

Limitation in intrinsic resources affects evolution of metabolic rate in a parasitic wasp.

Notre première étude a permis de mettre en évidence une capacité de lipogenèse dans nos populations iraniennes prélevées dans une région désertique. Cette lipogenèse implique qu'il existe des différences entre populations dans la limitation en ressources énergétiques. Nous avons cherché à déterminer si ces différences pouvaient avoir eu un impact sur l'évolution du taux de métabolisme (taux d'acquisition et d'allocation des ressources) et de sa norme de réaction ou si un taux de métabolisme faible a été sélectionné dans les zones désertiques, comme décrit dans toutes les autres études.

Geographical variations in the level of phenotypic plasticity: Does reproductive strategy or environmental variation explain it?

Il est admis que les espèces/populations synovogéniques sont plus plastiques que les proovogéniques dans l'allocation des ressources. En comparant les populations proovogéniques et synovogéniques de *L. boulardi* trouvées en Iran dans différents climats et une population proovogénique de *L. boulardi* avec une synovogénique de *L. heterotoma* prélevée dans un même climat, nous avons cherché à déterminer si ces différentes stratégies de maturation d'œufs diffèrent par leur plasticité à la température.

6- Présentation des modèles biologiques

A- Espèces étudiées

Durant cette thèse, j'ai essentiellement travaillé sur un hyménoptère parasitoïde de drosophiles, *Leptopilina boulardi* (Barbotin, Carton & Keiner-Pillault, 1979) (Hymenoptera: Eucoilidae). Il s'agit d'un endoparasite solitaire présent sur tout le pourtour méditerranéen, en Afrique tropicale, en Amérique du Sud et du Nord, et dans les Caraïbes. La limite nord de son aire de répartition se situe environ à 45°N mais celle-ci semble remonter, tout du moins en France. On le trouve dorénavant dans les environs de Lyon, alors qu'il n'y était pas présent il y a 30 ans (Carton 1984). Sa limite sud reste mal définie mais des spécimens ont été échantillonnés en Afrique du Sud et au Brésil (Fleury 2009).

L. boulardi s'attaque essentiellement aux larves de second stade (L2) de *Drosophila melanogaster* et *D. simulans*, voire de *D. yakuba* présente uniquement en Afrique (Dubuffet *et al.* 2008). Il existe une réaction immunitaire des larves de *D. melanogaster*, qui peuvent encapsuler et empêcher le développement du parasitoïde (Russo *et al.* 1996). Les femelles *L. boulardi* perçoivent la présence d'hôtes à longue distance via les odeurs laissées par les drosophiles adultes sur les substrats de ponte et la synergie des odeurs des drosophiles et des fruits dans lesquels les œufs de l'hôte ont été pondus (Couty *et al.* 1999). Sur le patch d'hôtes, la recherche d'hôte se fait par vibrotaxie et recherche aléatoire avec l'ovipositeur (Vet & Bakker 1985). Cette espèce est décrite comme strictement proovogénique (Kopelman & Chabora 1986).

Récemment, Varaldi *et al.* (2003) ont mis en évidence la présence chez certaines populations de *Leptopilina boulardi* d'un virus, baptisé Lbfv, qui induirait une augmentation du taux de superparasitisme. Ce virus, transmissible verticalement et horizontalement (entre une larve non infectée et une infectée présentes dans un même hôte), serait aussi responsable de la modification de certains traits. La production d'œufs est plus importante chez les femelles infectées, alors que leur activité locomotrice est fortement réduite et qu'aucune différence de longévité n'est observée (Varaldi *et al.* 2005).

Les souches de *L. boulardi* utilisées proviennent d'Iran (article 1, 4, 5) et des Pyrénées (article 2). Leur provenance et les méthodes d'échantillonnage sont précisées dans les articles concernés.

Figure 5. Femelles de *Leptopilina heterotoma* (gauche) et *Asobara tabida* (droite) en pleine oviposition

En parallèle, je me suis intéressé à deux autres espèces de parasitoïdes de drosophiles, *Leptopilina heterotoma* (Thomson, 1962) (Hymenoptera: Eucoilidae) et *Asobara tabida* (Nees, 1834) (Hymenoptera : Braconidae). Ce sont toutes deux des parasitoïdes de drosophiles synovogéniques, décrites comme incapables de synthétiser des lipides durant la vie adulte (Eijs *et al.* 1998, Ellers 1996*). Leptopilina heterotoma* se retrouve en Europe de l'Ouest et en Afrique du Nord et est capable de parasiter un grand nombre d'espèces de drosophiles (*D. subobscura*, *D. melanogaster*, *D. simulans*, *D. funebris*, *D. immigrans*). *Asobara tabida* est une espèce plus septentrionale présente de la côté méditerranéenne jusqu'au nord de la Scandinavie (Carton *et al.* 1986), mais également au Nord-Ouest du continent américain et au Japon. Cette espèce ne se développe que sur *D. subobscura* et *D. melanogaster*. La provenance des souches utilisées et l'élevage de ces espèces est détaillée dans l'article concerné (article 4).

B- Elevages

Hôte : Les *Drosophila melanogaster* utilisées comme hôtes proviennent d'une souche hollandaise élevée à l'université de Leiden, issue de croisements entre quatre populations naturelles prélevées en 1960 aux Pays-Bas. Durant cette thèse, les mouches ont été élevées en pots (h13cm*Ø6cm) placés dans des chambres climatiques (LMS 303) en conditions standard (25±0.1°C ; HR40±5 ; 12L :12D) sur un substrat d'Agar-sucre-levure-nipagine (antibiotique utilisé pour prévenir tout développement de moisissure). Une couche de levure vivante constituait le support de ponte et d'alimentation des larves (ainsi que le substrat d'agar), tandis qu'un morceau de papier absorbant était fourni aux larves comme substrat pour la pupaison.

Les larves de drosophiles proposées aux femelles parasitoïdes étaient « standardisées » afin de limiter les différences de taille des hôtes, facteur pouvant influencer les traits d'histoire de vie des parasitoïdes émergents. Pour ce faire, des drosophiles adultes étaient stockées à 12°C afin de bloquer la ponte, puis placées deux heures à 25°C dans un pot d'élevage (h13cm*Ø6cm) sur un substrat Agar-nipagine recouvert de levure vivante. Les œufs étaient ainsi pondus en grande quantité sur un court laps de temps, les stades et tailles de larves d'hôtes proposées aux parasitoïdes étant alors similaires.

Leptopilina boulardi :

Pour l'élevage standard, deux femelles et deux mâles de chaque souche étaient placés à 25°C durant 24 heures dans un pot sur substrat d'Agar-nipagine en présence de larves L2 de *Drosophila melanogaster* déposées sur une fine couche de levure. Durant le développement des larves de *Leptopilina,* les hôtes parasités étaient placés dans les mêmes conditions que les parasitoïdes adultes (chambre climatique LMS 303, 25±0.1°C ; HR 40±5% ; 12L : 12D). Les adultes étaient élevés en pot (h8cm*∅5cm) sur un substrat d'Agar-nipagine et alimentés avec du miel d'acacia dilué distribué *ad libitum*.

Chapitre 2.

Le climat, un facteur essentiel dans l'évolution des histoires de vie des parasitoïdes ?

Local adaptations of life history traits of a *Drosophila* parasitoid, *Leptopilina boulardi*: does climate drive evolution?

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Key words

Life-history theory, reproductive strategies, trade-offs, temperature, precipitation, resource distribution, parasitoids.

Abstract

1. Climate is an important source of selection on life histories, and local adaptations to climate have been described in several cline studies. Temperature is the main climatic factor that has been considered as an agent of selection, whereas other factors may vary with it, such as precipitation.

2. We compared life-history traits of five populations of *Leptopilina boulardi*, a *Drosophila* parasitoid, originating from contrasting climates. Referring to cline studies, we hypothesised shorter lifespan, earlier reproduction, and lower lipid content in populations from the hottest and driest areas if life histories have been selected in response to temperature and/or humidity.

3. Our results are opposite to these predictions. Females from humid and mild climates invested more in early reproduction and lived for fewer days than females from dry and hot areas, which were synovigenic (i.e. they matured additional eggs during adult life) and able to synthesise lipids during adult life.

4. We suggest that life histories are more adapted to host distribution than to climatic factors. *Drosophila* patches are more abundant in the humid area, allowing the parasitoids to spend less energy and time finding hosts. This may result in selection for early reproduction traded-off against longevity. In the hot and dry climate, females have to fly large distances to find host patches. Synovigeny, a long lifespan, lipogenesis, and high dispersal ability may be adaptive there. This is the first time that between-population differences in the ability to synthesise lipids have been described in parasitoids.

Introduction

Life histories evolve in response to environmental conditions (Stearns, 1992). In the last decade, climate as a selective factor has been well investigated as it can affect physiology and/or behaviour of organisms, particularly that of ectotherms. Genetic differences due to climate-dependent selection onlife-history traits have been shown in several studies of geographic variation, where populations originating from cold and hot climates have been compared in common garden experiments (e.g. Liefting *et al*., 2009). Comparative studies of populations of insect species, particularly Drosophilidae, at a continental scale or along an altitudinal cline have documented between-population variation of several but isolated life-history and morphological traits. For example, increase in thorax length (Dahlgaard *et al*., 2001) and decrease in knock-down resistance (Sørensen *et al*., 2005) with an increase in altitude have been recorded in *Drosophila buzzati*, while starvation resistance increases with increasing latitude in *Drosophila burchii* (Griffiths *et al*., 2005).

Environmental conditions do not only affect one or more traits in isolation but can affect the balance of allocation of resources to a large range of life-history traits. For example, development time generally decreases with increasing temperature but adult body size also decreases in a majority of insects studied (Sibly & Atkinson, 1994). Fecundity also increases with temperature whereas longevity decreases, as a trade-off occurs between these two traits (Nunney & Cheung, 1997). Thus reproductive allocation strategies and trade-offs can show clinal variation, e.g. an increase in early reproduction with altitude traded off against a shorter lifespan (Norry *et al*., 2006). Correlations between traits involve studying a large range of traits when interested in climate-dependent evolution of life histories and that consideration has to be taken into account in comparative studies.

Most studies considered only a temperature gradient to explain evolution of life-history traits but humidity and precipitation gradients that occur on a cline should also be explored. Desiccation resistance is the only life-history trait that has been considered as a result of differences in humidity (Karan *et al*., 1998; Gilchrist *et al*., 2008) but other traits may be under selection of humidity and precipitation, such as timing of egg-laying. Here we propose a study on local adaptations of a large range of life-history traits in a *Drosophila* parasitoid, *Leptopilina boulardi*, to understand how resource allocation strategies have evolved in response to climate. We compared

populations originating from a mild and humid area with populations from a very dry and hot desert area and a population from a cool environment but intermediate in precipitation. We considered temperature and humidity gradients as climatic factors that may result in adaptation of life histories.

As no formal theory exists about the impact of temperature on life histories, we used data from empirical studies to formulate hypotheses. The few cline studies that have been published can be used to make predictions about the impact of temperature on selection in several life-history traits in insects. In altitudinal and latitudinal clines studies, lifespan (Schmidt *et al*., 2005; Schmidt & Paaby, 2008) of *Drosophila melanogaster* females, the main host of *L. boulardi*, from colder environments exceeds that of females from hotter areas while their reproduction is delayed (Mitrovski & Hoffmann, 2001; Schmidt *et al*., 2005; Schmidt & Paaby, 2008). Moreover, a low wing loading [i.e. the ratio of fresh mass to wing surface that corresponds to the pressure exerted by the wings on the surrounding air (Gilchrist & Huey, 2004)] is thought to be selected under cool conditions to compensate the lower wing beat frequency at lower temperatures in insects (Unwin & Corbet, 1984). Compared with cold or mild environments, we can expect that lifespan of the parasitoid wasps from the hottest environment may be reduced. This shorter lifespan may have resulted in the selection of an early reproduction traded-off against lower lipid content at emergence as a trade-off generally occurs between these two traits in parasitic wasps (Ellers & van Alphen, 1997). Lipids have to be considered when investigating parasitoids life histories as they represent the main energetic resources allocated to survival and reproduction in parasitoids.

Importance of humidity in selection of life histories has been poorly investigated and no cline studies can be used to make predictions because only temperature has been considered as an explanative factor. However similar results of selection for the driest areas as in the hottest areas may be expected as lifespan may be reduced because of a high risk of desiccation and early reproduction may be adaptive in very dry environments where favourable reproductive periods are relatively short in comparison to humid environments.

To resume, compared with cold and dry or mild and humid environments, we may observe shorter lifespan, earlier reproduction, lower lipid content and higher wing loading in populations from the hottest and driest areas. We discuss our results in the

light of the above-mentioned predictions for the importance of temperature and humidity in the selection of life histories.

Material & Methods

Leptopilina boulardi (Hymenoptera: Figitidae) is a solitary endoparasitoid that mainly attacks *D. Melanogaster and D. simulans* larvae (Diptera: Drosophilidae) living in fermenting fruits. This species occurs principally in regions with a Mediterranean climate and in tropical Africa. *Leptopilina boulardi* females have been described as proovigenic (Kopelman & Chabora, 1986), i.e. they mature all their eggs before the start of their adult life.

Source of laboratory samples

Five populations of *L. boulardi* from contrasting climates (Table 1) were collected in Iran (Fig. 1) in July 2006 using twelve banana bait traps per site. Each open trap (i.e. a plastic container with a 3 cm diameter hole covered with a mesh with 2 mm openings) was colonised by five to twenty females and several hundreds of offspring were produced in each of them. *Leptopilina boulardi* and *L. heterotoma* were the only parasitoids and *Drosophila melanogaster* and *D. simulans* were the only fruit flies we found in our traps in all locations. From the offspring thirty females per population were taken to set up lab cultures. Two populations - Chalus and Seyakhal – originated from a humid and mild environment, in the coastal plain of the Caspian Sea where orchards, the main habitat for fruit flies, and fruit trees are abundant during a long season. Moreover, there are a lot of forests where *Drosophila* can be found in the area. Dorcheh and Zamankhan strains originated from a hot and very dry area, although the location of our traps was in the valley of a river fed by melting snow from the mountains that keeps also water throughout summer and allows for agriculture under irrigation in a narrow zone bordering the river. In this area, fruits are produced in few and distant orchards. These few orchards represent the only habitat where fruit flies are present as the remaining area is desert. The last strain - Sorkhabad - originated from the Alborz Mountains, with the coolest climate but intermediate in humidity and number of rainy days between Chalus/Seyakhal and Dorcheh/Zamankhan. These five localities have been chosen within a relatively small

area in comparison to continental-scales studies to compare populations with recent isolation (less than 100 km between Seyakhal, Chalus and Sorkhabad and between Zamankhan and Dorcheh, 400 km between Dorcheh/Zamankhan and Chalus/Seyakhal/Sorkhabad).

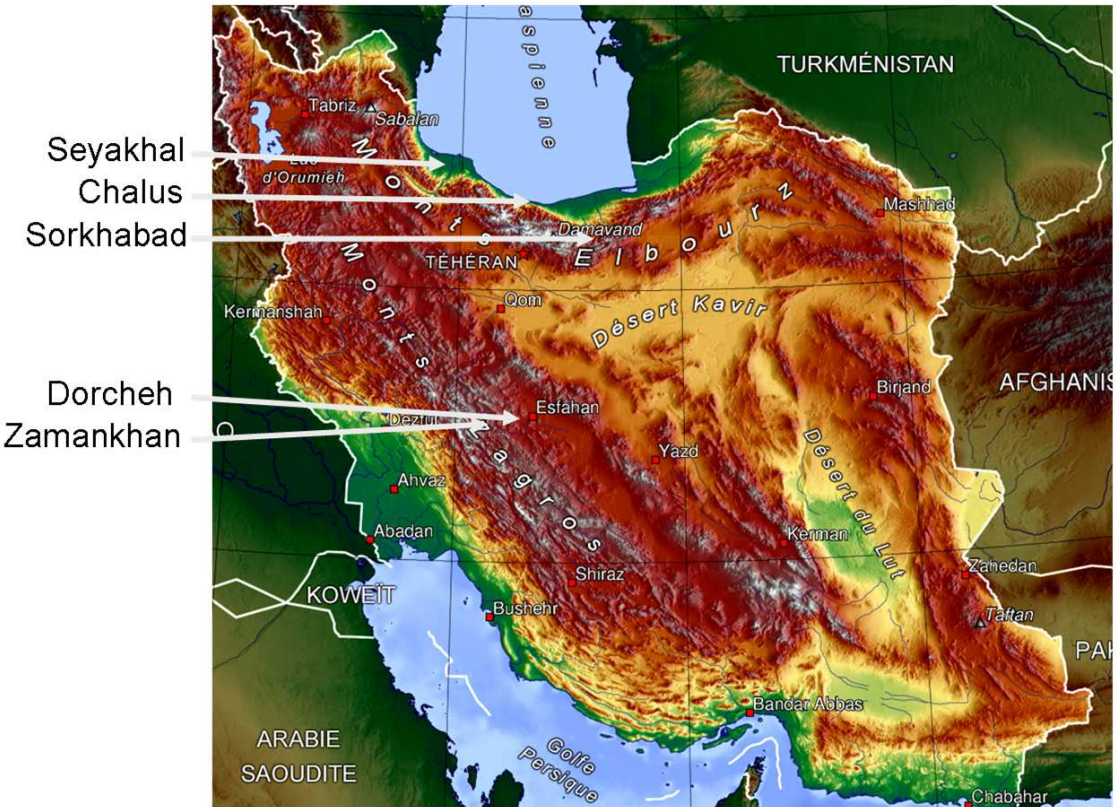

Figure 1. Map of Iran; the arrows indicate the five *Leptopilina boulardi* sampling sites.

Table 1. Climatic data recorded for the four sampled areas, from 1977 to 2005 for Chalus, 1955 to 2005 for Seyakhal and Zamankhan, 1993 to 2003 for Sorkhabad, 1951 to 2005 for Dorcheh (Source: Islamic Republic Of Iran Meteorological Organization). We considered the average of each parameter measured every day from April to September, which is the period of activity of *Leptopilina boulardi*. Thermal amplitude was calculated as the mean of the differences between maximum and minimum air temperature per month.

Sampling areas	Temperature (°C)	Thermal amplitude (°C)	Relative humidity (%)	Number of rainy days	Precipitation amount (mm)
Chalus	26.3	7.6	80	43.3	248
Seyakhal	26.4	9.8	79	56.7	433
Sorkhabad	24.4	16.7	44	28.6	105
Dorcheh	31.6	15.6	29.7	14.2	30.9
Zamankhan	28.6	19.2	36	13.8	55

We carried out between-populations crossings to ensure that all populations indeed belonged to the same species, *L. boulardi*. For each population, five crossings were performed with males of the other strains. We obtained fertile females in all crossings confirming the conspecificity of the populations, as only inseminated mothers can produce females in haplo-diploid species.

Cultures

Drosophila melanogaster used as hosts in the cultures originated from strains collected in the Netherlands in 1960. Ten *L. boulardi* females oviposited in separate jars for 48h in 100 to 120 second instar *D. melanogaster* larvae laid in a two hours period (similar size for every host) in a baker's yeast suspension. The parasitized larvae were reared at 25°C, 50% RH, 12L: 12D in glass jars on an Agar-Nipagine substrate. This temperature was chosen as it is the one at which a maximum of offspring is produced for all populations. Progeny of a same mother was separated in three groups. The first one was used for measurements of morphometric traits, number and volume of eggs and lipid content, the second one for the measurement of longevity and the last one to measure activity. Adults were fed with diluted acacia honey distributed *ad libitum*.

Presence of Lbfv virus.

Varaldi *et al.* (2003) described the presence of Lbfv viral particles in some *L. boulardi* populations and showed that this virus causes an increased tendency to superparasitize in infected females. These viral particles are also involved in changes in other life history traits such as egg load. We first checked the presence or absence of superparasitism to be sure that the virus could not explain variations in other life history traits between populations. We used a protocol similar to the one described by Varaldi *et al.* (2003). All the experiments were conducted at 25°C. Twenty females (24 to 48h old) originating from each population were isolated for 16 hours (from 6 pm to 10 am) with ten *Drosophila melanogaster* larvae (48h old) foraging in an arena consisting of a thin yeast spot spread over an agar layer poured into a 5 cm diameter Petri dish. After two days of incubation, *Drosophila* larvae were dissected and parasitoid eggs were counted to estimate superparasitism.

No self-superparasitism occurred in our experiments. We never found more than a single egg per parasitized larva in experiments with females from all five populations. There is thus no evidence for the presence of the virus Lbfv in these populations. Hence, Lbfv can not explain between-populations differences in egg load and other life history traits.

Life history traits

Morphometric traits, number and volume of eggs and lipid content were measured on twenty females from each population at 24 hours, five days and ten days after emergence except for Sorkhabad strain which had been lost before measurements at five and ten days of life. Females were placed in Eppendorf tubes, frozen in liquid nitrogen to stop all metabolic activity instantaneously and conserved at –80°C. These females constituted the first group of individuals.

Morphometric traits. After defrosting, fresh mass (FM) was determined with a microbalance (Sartorius M4 ±0.001mg) while left wing length was measured (±0.01mm) with the numeric image analysis software Pegasus Pro V4 under a binocular linked to a camera video (JVC KY-F). Wing loading was then calculated as the ratio of fresh mass to squared wing length. Wing loading corresponds to the pressure exerted by the wings on the surrounding air (Gilchrist & Huey, 2004). Thus the cost of transport is influenced in an important way by the wing area that supports the body mass (Starmer & Wolf, 1989). The lower the wing loading, the less costly the flight is.

Investment in early and lifetime reproduction. Females used for morphometric traits were placed in a drop of Ringer's solution on a microscope slide and dissected under a binocular (×40, Olympus SZX9). Eggs were removed from both ovaries and counted, while the rest of the female's body was conserved for later lipids extraction. After counting, eggs were placed under a microscope (×4, Olympus BH2) and photographed (Olympus Camedia C3040). Length (L) and width (w) of 30 eggs of each female were measured with the numeric image analysis software AnalySIS to calculate eggs volume (taken as a prolate spheroid volume: $V=4/3\pi Lw^2$). Investment

in reproduction was calculated as the result of number of eggs*volume of eggs. This parameter is a measure of energy invested into reproduction.

Lipid content. After eggs had been removed from all the females measured of different ages, we measured lipid quantity of the females using the protocol proposed by Vernon & Vannier (1996) and Terblanche *et al.* (2004). Wasps were dried at 40 °C during four days in an air oven and weighed to measure dry mass. Then parasitoids were left during two weeks in an Eppendorf tube containing 1 ml of chloroform/methanol solution (2:1) to extract lipids. Then females were again placed at 40°C during 24 hours and weighed again to measure lipid quantity and lipid content (=lipid quantity/lean dry mass). This lean dry mass (without eggs) was used as an indication of body size in our statistical analyses.

Longevity. This parameter was measured in the second group of wasps (n=20 per population). Females from each population were placed at 25°C on an Agar-Nipagine substrate and were fed with honey. The substrate was renewed every two weeks. Dead individuals were counted and removed twice a day, each morning and evening.

Activity. This parameter was measured in the third group of individuals (n=20 per population for Chalus, Seyakhal and Zamankhan strains). The behavioural data acquisition and analysis software Ethovision (Noldus Information Technology 1997) allowed us to record and analyze locomotive activity of parasitoids. Females were fed with honey during the first 24 hours of their adult life. Then they were isolated in Petri dishes (∅ 5 cm) thirty minutes before the start of the recording. For each run, four one-day old females belonging to the three populations were filmed simultaneously (camera Panasonic CCTV) in a windowless room during 24 hours: 12 hours with artificial light and 12 hours with red light only, simulating night.. The contrast between individuals and a white background was used to detect females and record their position twelve times per minute. The total distance covered and the average velocities were automatically calculated from the track records.

Statistical analysis

Variations in life history traits between populations.

Correlations between body size parameters (i.e. fresh mass, lean dry mass and wing length) and (1) reproductive parameters and lipid reserves for the first group and (2) longevity for the second group and (3) activity for the third group of individuals were examined for each population and for the complete data set.

ANCOVA and ANOVA were performed to analyze the effect of population and age on the different traits mentioned above. Post hoc tests were done to compare individuals of a same age from different populations and individuals of different ages within a population. Analyses of covariance (ANCOVA) were used when a correlation occurred between the trait tested and body size to remove any effect of size from the measurement and ANOVA tests were used when no correlation was detected.

The number of eggs ($r^2 = 0.6$, $P < 0.0001$) and lipid quantity ($r^2 = 0.64$, $P < 0.0001$) increased with lean dry mass but the volume of eggs and activity were neither correlated with lean dry mass ($P > 0.05$) nor fresh mass ($P > 0.5$) respectively. No correlation was found between longevity and dry mass in any population ($P > 0.05$).

Thus we used ANCOVA to examine differences in number of eggs, in investment in reproduction and in lipid quantity by incorporating lean dry mass as a covariate. ANCOVA was also used to test differences in squared wing length using fresh mass as a covariate. This test was performed to compare wing loading. Differences in the volume of eggs and in activity (i.e. total covered distance and velocity) were analysed with ANOVA. Differences in activity between day and night for a population were tested with Student's t tests.

The survival package provided by R software was used to test differences in longevity between populations, using a Weibull distribution.

Climatic factors in selection of life-histories.

We made Generalized Linear Models using the global dataset of populations to test for correlations between all life history traits and climatic factors: air temperature, thermal amplitude, relative humidity, number of rainy days and precipitation. Climatic factors were included in the base model and removed one by one to obtain the minimum adequate model. The selection was done on the basis of the AIC

criterion (Akaike Criterion, Akaike 1974). P-values reported are those of the last model that contains the variables.

All analyses were carried out using R software version 2.5.0 (R Development Core Team, 2007).

Results

Variations in life history traits between populations.

Wing loading (Fig. 2). Females from Chalus, Seyakhal and Sorkhabad had a similar wing loading. Dorcheh and Zamankhan females had a similar wing loading but lower than the other populations (ANCOVA, F= 48.98, df = 5, P < 0.0001). Wing loading did not differ between the different ages for Chalus, Seyakhal and Sorkhabad populations but it increased in Zamankhan (ANCOVA, F = 48.25, P < 0.001) and Dorcheh (ANCOVA, F = 29.4, P < 0.001) populations between five days and ten days of adult life as their fresh mass increased.

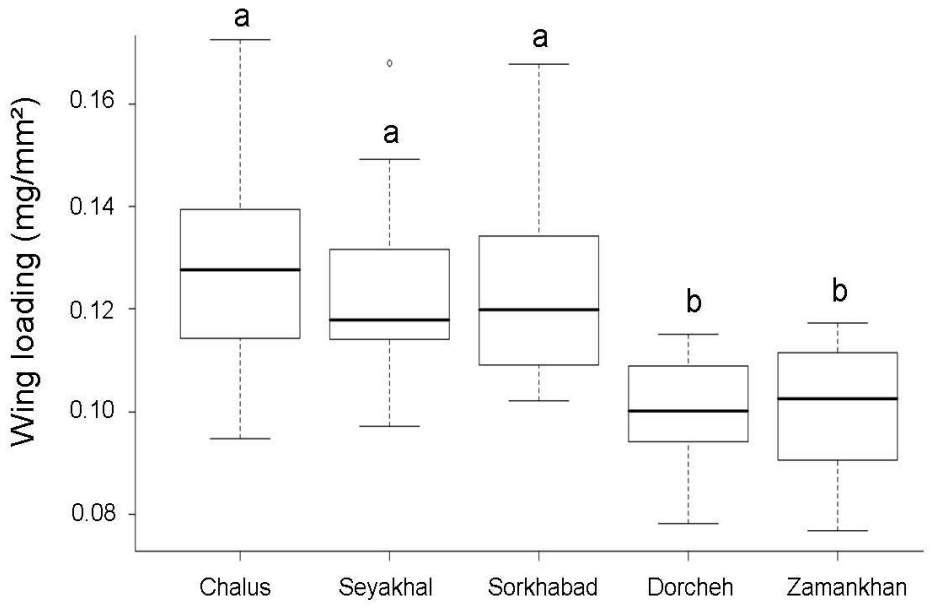

Figure 2. Wing loading measured at emergence for Chalus, Seyakhal, Sorkhabad, Dorcheh and Zamankhan females. Different letters indicate significant differences of wing loading between populations (n = 20 per population, P < 0.05). Error bars: ± SE.

Investment in early and lifetime reproduction (Fig. 3). For the same lean dry mass, females originating from Chalus and Seyakhal emerged with more eggs than Dorcheh and Zamankhan females (ANCOVA, $F = 83.36$, $df = 5$, $P < 0.0001$) (Fig 3A, <24 hours). Sorkhabad individuals emerged with an intermediate number of eggs.

No change in number of eggs was observed in Chalus females (ANOVA, $F = 0.008$, $df = 2$, $P = 0.96$) or Seyakhal (ANOVA, $F = 0.005$, $df = 2$, $P = 0.99$) between 24 hours and ten days of adult life. Dorcheh females increased the number of eggs in their ovaries (ANOVA, $F = 12.67$, $df = 2$, $P < 0.001$) during the first five days of adult life ($P = 0.012$) but no change was observed between five and ten days ($P = 0.99$). Zamankhan females increased the number of eggs in their ovaries during the first ten days of adult life (ANOVA, $F = 6.7$, $df = 2$, $P < 0.001$) (Fig. 3A).

The Chalus wasps had smaller eggs than the other populations (ANOVA, $F = 2.69$, $P = 0.03$) which did not differ between each other. We did not observe any difference in the volume of eggs in any of the populations between the three ages (ANOVA, $P > 0.6$). We found a negative correlation between the number and the volume of eggs in Chalus females ($r^2 = 0.53$, $P < 0.001$). This correlation was not found in any other population ($P > 0.05$).

Chalus and Seyakhal females invested more in early reproduction (i.e. number of eggs*volume of eggs, corrected by lean dry mass) than Dorcheh and Zamankhan females while Sorkhabad individuals had an intermediate investment in early reproduction (ANCOVA, $F = 4.078$, $P < 0.0001$). (Fig. 3B).

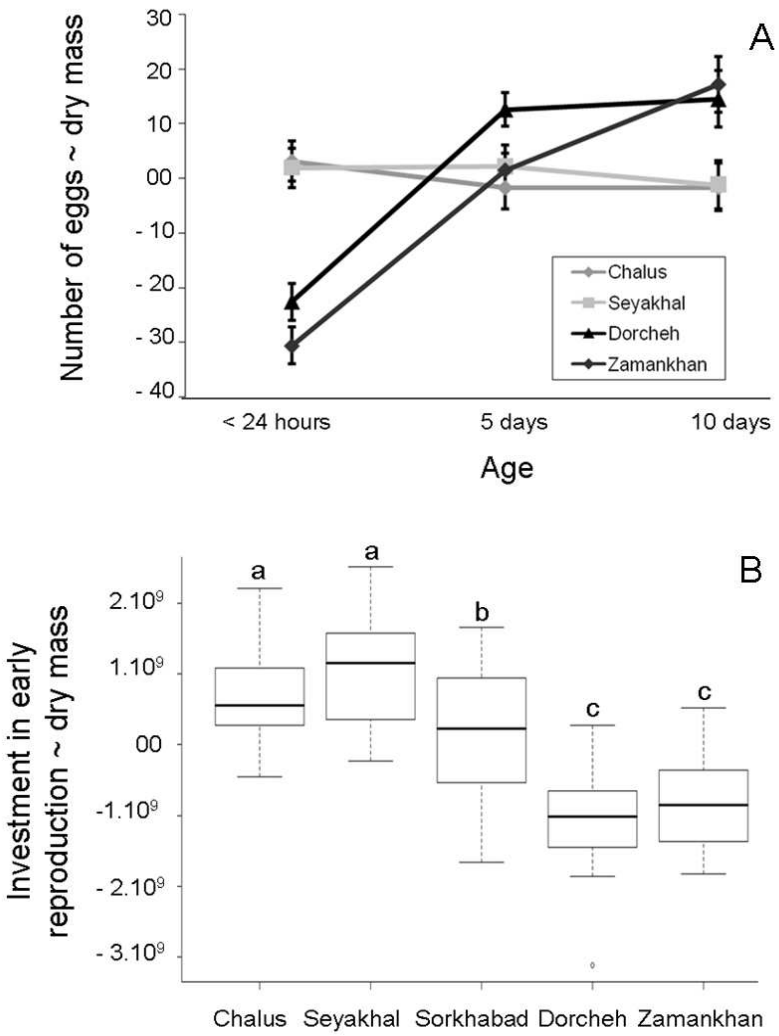

Figure 3. (a). Plot of residuals of models comparing mean number of eggs at 24 hours, 5 days and 10 days of adult life with lean dry mass as covariate. A model was established for each population. The higher the residuals, the more females of a same lean dry mass have eggs in their ovaries (n = 20 per age per population). Error bars: ± SE. (b) Plot of residuals of a model comparing investment in early reproduction (=number of eggs x volume of eggs) between populations with lean dry mass as covariate. The model was established with the global data set. The highest the residuals, the more females of a same lean dry mass invested in reproduction. Different letters indicate significant differences of investment in reproduction between populations (n = 20 per population, $P < 0.05$). Error bars: ± SE.

Lipid content (Fig. 4). Chalus, Seyakhal and Sorkhabad populations had similar lipid contents and females of these populations emerged with a higher lipid content than Dorcheh and Zamankhan females (ANCOVA, $F = 48.98$, $P < 0.0001$) (Fig. 4A).

No change in lipid content was observed for Chalus (ANCOVA, F = 0.009, P = 0.97) and Seyakhal females (ANCOVA, F = 0.007, P = 0.99) during adult life. In contrast, females of the Dorcheh (ANCOVA, F = 9.27, P < 0.001) and Zamankhan populations (ANCOVA, F = 11.28, P < 0.001) increased their lipid content considerably during the first ten days of adult life (Fig. 4B).

Within populations, negative correlations were found for all populations between number of eggs and lipid content (n = 20, r^2 = (0.34-0.63), P < 0.05).

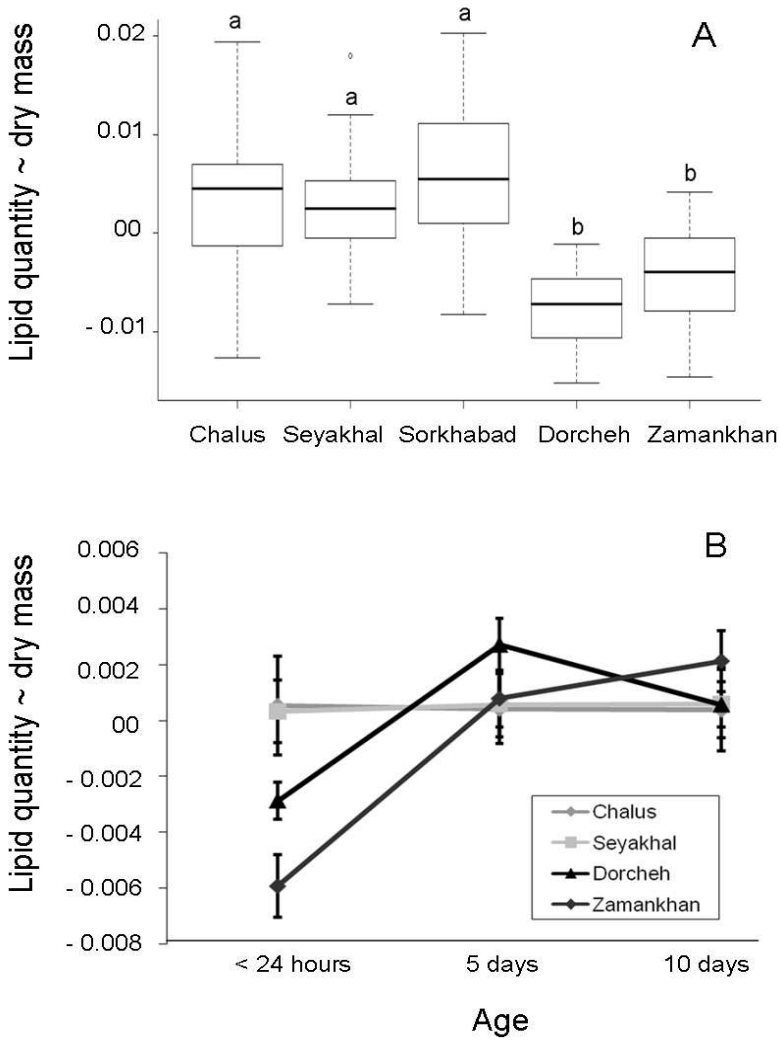

Figure 4. (a) Plot of residuals of a model comparing lipid quantity between populations with lean dry mass as covariate at emergence. The model was established with the global data set. The highest the residuals, the more females of a same lean dry mass have lipid reserves. Different letters indicate significant differences of lipid content between populations (n = 20 per population, P < 0.05). Error bars: ± SE. (b) Plot of residuals of models comparing lipid quantity at 24 hours, five days and ten days of adult life with lean dry mass as covariate. A model was established for each population. The higher the residuals, the more females of a same lean dry mass have lipid reserves. Error bars: ± SE. (n = 20 per age per population).

Longevity. Seyakhal females had the shortest lifespan (longevity = 11.5 ± 1.47 days, mean ± SE) while Zamankhan females lived significantly longer (longevity = 21.7 ± 0.82 days) than females of any other population (Survival analysis, Chisq = 65.46, P < 0.005). Dorcheh females (longevity = 20.2 ± 0.7days) lived longer than Sorkhabad (longevity = 18.7±0.91days) and Chalus individuals (longevity = 17 ± 1.7 days) that had a similar longevity.

Activity (Fig. 5). Seyakhal and Chalus females covered significantly shorter distances than Zamankhan females during the experiments (ANOVA, F = 8.497, df = 2, P < 0.05). The same pattern was found for velocity (ANOVA, F = 4.522, df = 2, P < 0.05). Moreover, Chalus (Student's test, t = 2.18, df = 19, p = 0.032) and Seyakhal (Student's test, t = 2.79, df = 19, p = 0.015) females were more active during day while Zamankhan individuals were more active during night (Student's test, t = -4.95, df = 19, P < 0.005).

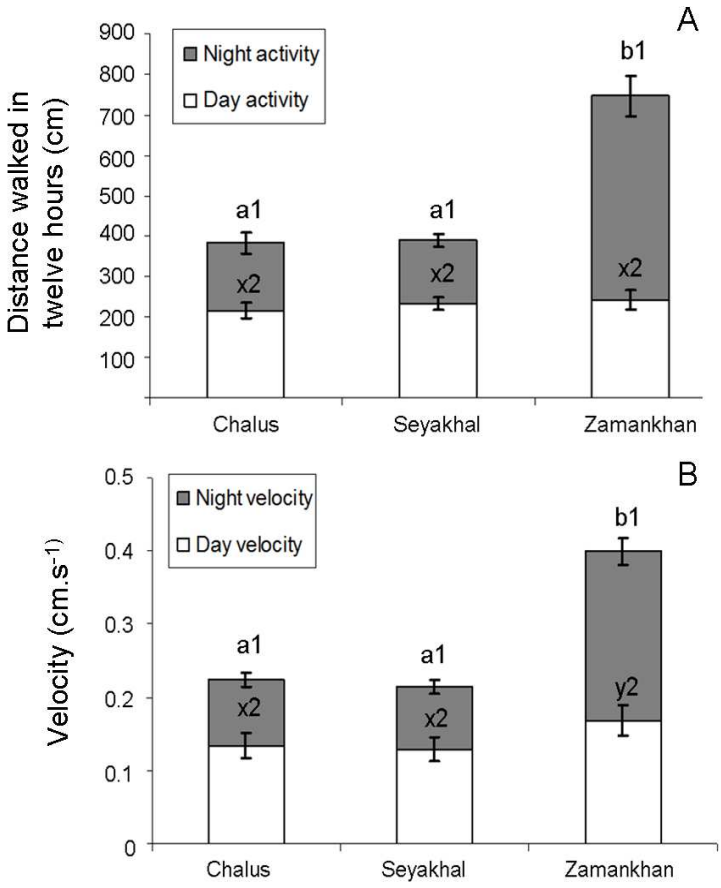

Figure 5. (a) Mean distance walked during twelve-hours day and night recordings, measured with Ethovision software. Different letters indicate significant differences of activity between populations. Different numbers mean significant differences of activity between day and night recordings for a single population (n=20 per treatment perpopulation, P < 0.05). Error bars : ± SE. (b) Mean velocity during twelve-hours day and night recordings, measured with Ethovision software. Different letters indicate significant differences of velocity between populations. Different numbers indicate significant differences of velocity between day and night recordings for a single population (n=20 per treatment per population, P < 0.05). Error bars : ± SE.

Climatic factors in selection of life-histories (Fig. 6). Climatic factors explained patterns for wing loading ($t = 2.625$, $P < 0.0001$) and investment in early reproduction ($t = 7.73$, $P < 0.0001$), lipid content ($t = 4.8$, $P < 0.0001$), and longevity ($t = 10.95$, $P < 0.001$). For these life history traits, the minimum adequate generalized linear models excluded air temperature and thermal amplitude as explanatory factors but included relative humidity, number of rainy days and amount of precipitation.

Investment in early reproduction (Fig. 6A), lipid content at emergence, and wing loading increased with these factors but longevity decreased (Fig. 6B).

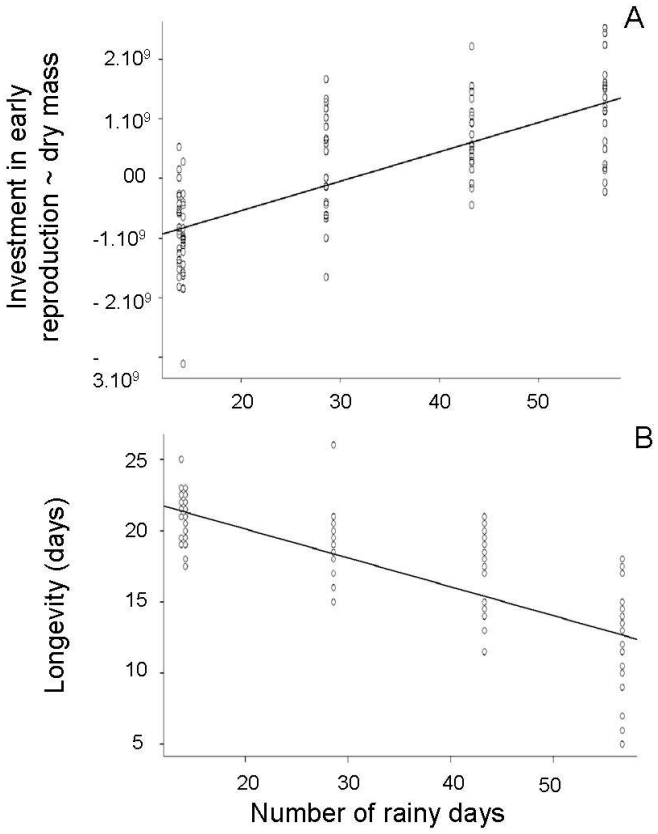

Figure 6 (a) Plot of a generalized additive model realized on investment in early reproduction with lean dry mass as covariate and number of rainy days as an explanatory factor. Relative humidity, number of rainy days and precipitation amount had to be included as explanatory factors to obtain the minimum adequate model for this life history trait. Here we represent the plot of a model on investment in early reproduction with the more significant factor in our model, the number of rainy days. (b) Plot of a generalized additive model realized on longevity with number of rainy days as an explanatory factor. Relative humidity, number of rainy days and precipitation amount had to be included as explanatory factors to obtain the minimum adequate model for this life history trait. Here we represent the plot of a model on longevity with the more significant factor in our model, the number of rainy days.

Discussion

Compared to females from mild and wet environment (Chalus and Seyakhal) and females from cool environment and intermediate in precipitations (Sorkhabad), females from hot and very dry environments (Dorcheh and Zamankhan) invested less in early reproduction and had a longer lifespan, lower lipid contents and wing loading during the first twenty-four hours of adult life. Females from the coolest climate but intermediate in precipitations and relative humidity (Sorkhabad) invested an intermediate amount of energy into early reproduction and longevity. Our results are not consistent with other latitudinal cline studies on adaptations of life history traits to temperature (e.g. Schmidt *et al.*, 2005, Schmidt & Paaby, 2008, Mitrovski & Hoffmann, 2001, Gilchrist & Huey, 2004).

Our parasitic wasps were collected in a relatively small area in comparison to the above cited latitudinal cline studies on Drosophilidae. The mean temperatures of our coolest and hottest climate were respectively 24.4°C and 31.6°C. Thus, our temperature range was quite smaller but on average warmer than in the other cline studies. This could possibly explain why we did not obtain similar results. Moreover, only *Drosophila* species were studied until now and parasitoids may respond differently to selection by temperature. Yet, one would expect that temperature should have similar effects in all ectotherms as it directly affects their physiology.

We therefore think that it is more likely that differences in life histories between our populations result from selection by another environmental factor than temperature. We considered precipitation and humidity in our study as possible agents of selection and found a strong link between number of rainy days/ precipitation amount and life history traits (i.e. investment in early reproduction, longevity, wing loading and lipid reserves). This result suggests that precipitation may be more important than temperature in selection of life-histories.

To our knowledge, no evidence of direct effect of humidity on selection of life history traits has been shown except for desiccation resistance (Gilchrist *et al.*, 2008, Karan *et al.*, 1998), but patterns for this trait are not really clear. Other studies on a humidity-dependent selection are needed to understand if this climatic factor may directly affect other life history traits than desiccation resistance.

A difference in climate involves more than only the abiotic environment as it may also affect the biotic environment e.g. interspecific interactions in the community or distribution of resources. We did not collect any other parasitoid species than *L. boulardi* and *L. heterotoma* in all locations so there is no evidence that differences in interspecific competition may explain differences in life history traits. Moreover, it is unlikely that variation in host species may explain differences between populations as we only found *D. melanogaster* and *D. simulans* in our traps. Our sampling areas differed in climate but also in landscape and biotic environment. Water availability determines fruit distribution -the main source of food for *Drosophila melanogaster*- and therefore laying opportunities for parasitoids. In the mild and humid area (Chalus and Seyakhal), there is a lot of precipitation during the whole season so fruits, and therefore *Drosophila* are common throughout the season. In the desert area (Dorcheh and Zamankhan), the lack of precipitation during summer restricts the production of fruits to few and distant areas under irrigation. Here, subsequent populations of parasitoids have to move between crops of different fruits, because when they emerge as adults, the season for the fruit in which they developed is over. This necessitates long flights in search of host habitats. Thus we will mainly explain our results on life history traits in the light of a selection in response to host distribution as it is affected by humidity.

Reproductive strategies and longevity.

We found a high investment in early reproduction of females from mild and wet environments (Chalus and Seyakhal), correlated with a shorter lifespan. These results suggest that females from these populations are pro-ovigenic i.e. they do not mature eggs during adult life but females were not allowed to lay eggs. According to models developed by Ellers *et al.* (2000) and Ellers & Jervis (2004), host distribution is the most important factor resulting in the selection of pro-ovigeny and may explain our results: in environments with small distances between host patches and a high encounter rate with hosts, selection results in the allocation of energy to early reproduction (i.e. pro-ovigeny) at the cost of a shorter lifespan.

Sorkhabad females originate from the coldest area where rainy days and the precipitation amount are intermediate between Chalus/Seyakhal and Zamankhan/Dorcheh. Investment in early reproduction and longevity were both intermediate in Sorkhabad females. The results for this population supports the fact

that precipitation and not temperature is important in selection on reproductive strategies.

Females from the desert area (Dorcheh and Zamankhan) invested less in early reproduction but lived longer than other populations. Our results also provide evidence that *Leptopilina boulardi* is not a completely pro-ovigenic species as suggested by Kopelman & Chabora (1986): we observed an increase of more than 50 eggs during the first ten days of adult life in Dorcheh and Zamankhan females. Females from these populations were synovigenic (i.e. they still mature eggs during adult life) and lived longer than those of other populations. Synovigeny and increased lifespan allow a plastic response in an environment where females have to move over large distances to find hosts and are time-limited (Ellers & van Alphen 1997). They can adjust egg maturation to the laying opportunities and trade current for future reproduction. This could explain the synovigeny in the Dorcheh and Zamankhan populations. Our results suggest that pro-ovigenic and synovigenic populations may occur within the same parasitoid species. Such variation in reproductive strategies between populations has never been described in parasitoids. Therefore, we have to confirm the pro-ovigeny of Seyakhal and Chalus females by providing them hosts and checking if laying opportunities lead to the maturation of additional eggs during adult life (i.e. synovigeny) or not (strict pro-ovigeny) in these populations.

Variations in life history traits between populations suggested that a trade-off occurs between longevity and investment in early reproduction. However, this trade-off was not found for females after five days of adult life because Zamankhan females, that lived longer, matured additional eggs while females from mild and humid area did not. Females were not limited by carbohydrate resources in our fecundity experiments, and thus could allocate more of their lipid reserves to reproduction. In nature, female parasitoids from the desert area are food and time limited because they have to travel long distances to find patches of hosts and sources of food. Thus a trade-off may occur between longevity and realised fecundity that we did not detect here because females were not allowed to lay eggs and were fed *ad libitum*.

Lipid content. In parasitoids, a longer lifespan is often associated with higher lipid reserves at emergence (Ellers, 1996; Rivero & West, 2002) when initial egg load is traded-off against fat reserves (Ellers & van Alphen, 1997). Indeed we observed a

negative correlation between initial number of eggs and lipid content for the five populations studied. A trade-off between fecundity and lipid reserves exists during larval development where resources are limited by the amount available from the host. Surprisingly, females from the dry and hot area lived longer than females from the other areas but emerged with smaller lipids reserves. Parasitoids were thought to be unable to synthesize and accumulate lipids during adult life and to depend only on lipids accumulated during larval development (Giron & Casas, 2003; Visser & Ellers, 2008). However Visser *et al.* (2010) recently described lipogenesis in some parasitoid species and suggest that "the wide range of host species in which generalists are able to develop may impede effective host manipulation and could have resulted in regaining of lipogenic ability in generalist parasitoids". In opposition to the authors who did not find any lipogenesis in *L. boulardi*, we found a strong increase in lipid quantity in this species for females from desert areas during the first ten days of adult life, evidence that adult lipogenesis occurs in these populations. This is the first evidence that between-populations differences in the ability to synthesize lipids occur in a parasitoid species. Other factors than hosts range may result in existence of lipogenesis in parasitoids, such as host distribution. Lipogenesis may be adaptive in desert areas where females need lipids to fly over large distances and mature eggs once they have found a lot of laying opportunities. Moreover, higher lipid content may limit water loss in this dry climate (Hadley, 1994). The observed adult lipogenesis could explain the longer lifespan and increased egg maturation observed in these females.

Dispersal ability and locomotor activity. Wing loading was lower in females from hot and dry areas than in females from other environments. This reduced wing loading may facilitate flight (Gilchrist & Huey, 2004) in an environment where females have to move over large distances to find patches of hosts. Greater dispersal ability during the first days of life may compensate for the low number of hosts encounters and be selected because females can search for patches of hosts at lower cost in desert areas.

Flying over large distances to find hosts is an energy demanding activity (Ellers *et al.*, 1998). In a humid environment like that in Chalus or Seyakhal, females do not have to move over such large distances as in a desert environment. In the latter environment,

flying, which was not allowed in our experiment, to find laying opportunities may result in a decreased egg maturation and/or longevity.

Zamankhan females were also more active than females from mild and wet environment. This high activity may have been selected in an environment where females have to move a lot to find hosts in distant patches. Moreover Zamankhan females were more active during the night while females from Chalus and Seyakhal were more active during the day. Avoidance of extreme temperatures in Zamankhan during the day is likely to be adaptive in this desert area. The nocturnal activity of Zamankhan females is the only trait that supports a direct effect of temperature on the evolution of life history traits.

Conclusion

Females from the desert area differed considerably from females originating from the mild coastal region, but these populations were also geographically distant and this separation by distance has allowed local adaptation and caused differences in life histories between populations as a result of selection by the environment.

Differences in temperature and thermal amplitude did not explain our results, but precipitation did. The influence of precipitation on fruit availability, the main nutritional resource for hosts, could explain why this climatic factor is more linked to life history traits than others. Our results, especially synovigeny and lipogenesis, strongly support the hypothesis of selection based on distribution of resources and suggests that host distribution, depending on precipitation, is more important in selection of life history traits of parasitoids than temperature.

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Life-history trade-offs' shift in response to community changes: the case of the Drosophila parasitoids from the Rhône Valley

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Abstract

Life-history traits are linked to fitness and are influenced by both biotic and abiotic environmental factors. Organisms should display an optimal life-history in a given environment, within the limits of certain internal constraints. Changes in a parasitoid community are expected to influence life-history traits, and consequently modify trade-offs between maintenance, reproduction and dispersal. In this study, we investigated the impact of a community change (the presence of an invasive species) on the life-history traits of *Drosophila* larval parasitoids in the Rhône valley in France. Two native species, *Leptopilina heterotoma* and *Asobara tabida* are present all along the valley while a third invasive species, *L. boulardi*, is slowly extending its distribution towards the North of the valley. This invasive species being competitively superior and bringing high levels of parasitism of *Drosophila* larvae, we expect life-history to be different in southern populations where mortality is likely to be higher. As predicted, fecundity is higher in southern populations for both native species, coupled with higher dispersal ability in *A. tabida*. However, only *A. tabida* shows a shorter longevity as predicted by the trade-off between fecundity and longevity. Female *L. heterotoma* use a different mechanism to be able to invest more in fecundity: they have a lower metabolic rate consuming less lipids, coupled with adult lipogenesis.

Introduction

Life-history refers to a set of strategies including behavioral, physiological and anatomical traits (Ricklefs & Wikelski 2002), linked to fitness and strongly influenced by natural selection. According to the life-history theory (Roff 1992, Stearns 1992), individuals display an optimal life-history in a given environment. However, all possible combinations of life-history traits do not occur in nature due to the existence of internal trade-offs: available resources are allocated preferentially to some traits to the detriment of others. The most classic trade-off is between maintenance and reproduction and has been documented for several species (Ellers, van Alphen & Sevenster 1998; Therrien *et al.* 2008; Blomquist 2009): a higher investment in fecundity usually translates in a lower investment in longevity. Dispersal can also be traded-off against maintenance or reproduction (Roff & Fairbairn 1991; Zera & Denno 1997; Hughes *et al.* 2003; Gu *et al.* 2006).

The influence of environment on life-histories results from the effect of both abiotic and biotic variables (Stearns 2000). Abiotic factors influences reproduction timing and longevity through environmental harshness (risks of desiccation, low precipitation, strong wind and low temperature) in *Drosophila buzzatii* (Norry *et al.* 2006). Similarly, season length influences life-history traits in the water strider *Aquarius remigis* (Blanckenhorn & Fairbairn 2002). Biotic factors such as community structure can also influences life-history traits such as virulence of the parasitoid *Asobara tabida* and resistance of its *Drosophila* hosts (Kraaijeveld & Godfray 1999). Most of these abiotic and biotic variables are affected by the current change in climate occurring all over the globe (IPCC 2007). As a consequence, species' area of distribution and densities are modified, and life-history traits get adapted to the new climatic conditions (Gienapp *et al.* 2008, Lepetz *et al.* 2009).

The impact of climate change on life-histories is two-fold: there is a direct impact from the modification of abiotic variables and an indirect impact from changes in biotic variables (Walther *et al.* 2002, Gienapp *et al.* 2008, Lepetz *et al.* 2009). In the Rhône Valley in France, the larval solitary parasitoids of *Drosophila* represent an adequate system to study the adaptation of life-histories to changes in the composition of communities following climate change. North of this area, the community is mainly composed of the solitary endoparasitoids *Asobara tabida* Nees and *Leptopilina heterotoma* (Thompson). In the South, a third parasitoid species, *L. boulardi* (Barbotin *et al.*), is present and dominant (Fleury 1993, Fleury *et al.*, 2004), and is spreading northwards. *Leptopilina boulardi* is competitively superior to both native species, i.e. when multiparasitism occurs, the emerging parasitoid is

usually *L. boulardi* (Fleury *et al.* 2000). In addition, it has been shown that southern *Drosophila* community, where *L. boulardi* is present, is exposed to a higher level of parasitism from all parasitoid species than northern community (Fleury *et al.* 2004), most likely bringing decreasing host availability. The two native parasitoid species could thus experience higher interspecific competition in the southern areas, invaded by *L. boulardi*, than in the North where *L. boulardi* is absent or recently spread.

In this study, we examined the adaptation of parasitoid life-histories to changes in the composition of the *Drosophila* parasitoid community in the Rhône Valley. To reduce the confounding effect of abiotic factors, the present study was conducted on a 45 km zone around the front of progression of *L. boulardi*, near Lyon. For each native species, *A. tabida* and *L. heterotoma*, a population located North from the study zone, where *L. boulardi* was first detected two years before the sampling were conducted, was compared with a population located in the South where *L. boulardi* first arrived 11 years before the samplings.

Based on Price's balanced mortality hypothesis (Price 1974), stating that organisms submitted to high levels of juvenile mortality should have a high fecundity, we can expect, for both native species, a higher fecundity in the populations from the South, where higher mortality is likely to occur (directly or indirectly) because of the presence of *L. boulardi*. In addition, because lipids are the main energy resource for parasitoids, and are used for survival, egg production and dispersal (Ellers 1995, Ellers & van Alphen 1997, Giron & Casas 2003, Bernstein & Jervis 2008, Jervis, Ellers & Harvey 2008) this higher fecundity in females from the South should be traded-off against a shorter lifespan than females from the North.

Although this trade-off between fecundity and longevity has been observed in *A. tabida* (Ellers *et al.* 1998), it has not been shown for *L. heterotoma* (Ris 2003). This study will investigate this trade-off in more details, by measuring more traits related to maintenance (not only longevity, but also lipid content and metabolic rate) and reproduction (egg load at emergence and potential fecundity in addition to egg size), but also traits related to dispersal (wing loading and locomotor activity). We also expect that females from the south, expected to have a higher fecundity, would gain by being able to disperse further and being more active to find suitable hosts in an environment with lower host availability, but at a maintenance cost. In contrast, females from the north would have lower dispersal abilities.

Material and methods

Study sites

For both *A. tabida* and *L. heterotoma*, two populations were collected 45 km apart on a North-South transect in the Rhône valley. Insects were collected in October 2008 using banana bait trapping. *Asobara tabida* was collected in Anse (45°56'26.05"N, 4°21'18.40"E; altitude 170 m) and Pacalon (45°32'24.55"N, 4°47'29.31"E; altitude 299 m); *L. heterotoma* in Saint-Bernard (45°53'25.80"N, 4°46'41.56"E; altitude 170 m) and Seyssuel (45°33'34.76"N, 4°47'29.31"E; altitude 216 m). In the northern localities (Anse and Saint-Bernard), *L. boulardi* was first detected in 2006 whereas in the southern localities (Seyssuel and Pacalon), *L. boulardi* was found in 1997 (R. Allemand, unpublished data).

Climatic data

To take into account the confounding effect of abiotic factors, we compared climatic data (mean monthly temperature and mean monthly precipitation) provided by Météo France for nearby sites from 2004 to 2009: in Reventin (near the southern populations, 45°28'42"N, 04°48'36"E; altitude 295 m) and in Villefranche-sur-Saône (near the northern populations, 45°59'18"N, 04°43'24"E, altitude 212 m).

Rearing

Parasitoids were reared on a *D. subobscura* population collected in the Netherlands at the end of the 1980 decade. The very low resistance of this host to parasitoid attacks (Eslin & Doury 2006) enables to obtain a maximal number of parasitoids per generation. Hosts and parasitoids were reared at 20°C and 60% r.h. For the hosts rearing, adults were allowed to lay eggs in vials (H: 13 cm, ø 6 cm) containing a nutritive medium Agar-Kalmus-sugar-Nipagine topped with a small dome of living yeast.

For the parasitoid rearings, about 60 4d old (second instar) host larvae were put in vials (H: 8 cm, ø 5 cm) containing 1 to 1.5 cm of Agar-Kalmus-Nipagine medium covered with a thin layer of living yeast. Two males and two females, aged of 7 to 15 days, were then introduced in the vials for 24 hours. The hosts were reared until the emergence of adult hosts

and parasitoids. Adult parasitoids of both sexes were kept in vials with agar medium and nourished with 10% diluted honey.

General methods

All experiments were conducted at 20°C, 60% r.h., and L16:D8. Every trait was measured on females from the two populations for both species. Individuals were randomly distributed in the different measurements. For each individual used (except for the individuals on which metabolic rate and wing loading were measured, for which the fresh mass was taken), the length of the left hind tibia was measured with the numeric image analysis software Pegasus Pro V4 under a binocular (x3.15, Olympus SZ-6045TR) linked to a video camera (JVC KY-F). The tibia length was chosen as an estimation of the size of the individual (Nicol & Mackauer 1999).

Maintenance traits

Longevity

Longevity without food was measured (respectively n=54 and n=51 for the north and south population of *A. tabida*, and n=66 and n=61 for *L. heterotoma*). After emergence, the females were allowed to mate and kept into a vial containing a substrate of Agar-Kalmus-Nipagine providing water. Mortality was checked every day.

Lipid content

The amount of lipids in the body of virgin unfed females aged <2 h was measured using a colorimetric method with chloroform-methanol extraction and using Vanillin reagent (Giron *et al.* 2002) (respectively n=24 and n=21 for the north and south population of *A. tabida*, and n=25 for each population of *L. heterotoma*).

Metabolic rate

The metabolic rate of parasitoids was measured by flow-through respirometry as the quantity of CO_2 rejected per hour at a temperature of 20°C. Seven day old females were tested during the photophase (from 10.00 to 17.30 h) (n=21 for each population of each species). A mass flow valve controller (MFC-2) maintained constant flow rates. Ambiant air was drawn and

CO_2 and water were scrubbed with a Drierite-Ascarite column. Females were placed separately in small cylindrical chambers and their CO_2 production was measured with an infra-red CO_2 analyser (CA-10A Carbon Dioxide Analyzer). Four 5 minutes recordings were performed per individuals with a lag of 85 minutes between two successive recordings. Data were automatically transformed by a program recorded in Expedia software (Sable Systems, Berlin) in order to transform the measures from ppm to mL- CO_2 per hour, taking into account the flow rate. Metabolic rate was calculated as the average of the three last recordings.

Reproductive traits

Initial reproductive investment

Virgin females aged <2 h were frozen at -80°C and dissected under a binocular (x4, Olympus BH2) (n=25 for each population of each species). Egg load at emergence was measured by counting the mature eggs present in the ovarioles. After counting, 30 eggs per females were photographed (Olympus Camedia C3040) and the length (L) and width (w) measured with the numeric image analysis software ImageJ 1.14 to calculate egg volume. For *A. tabida*, the volume (V) of eggs was calculated as the volume of two identical cones attached by their bases: $V= 1/12\pi Lw^2$. The eggs of *L. heterotoma* were considered as prolate spheres with a volume of $V=4/3\pi Lw^2$. The mean volume was calculated for each female, and used for analysis.

Potential fecundity

The following experiment was designed to estimate the number of eggs matured by a female throught her life, hereafter defined as potential fecundity. All hosts used for this experiment were 4 d old L2 *D. subobscura* larvae.

Mated females were kept in vials with food but without hosts until they reached 7d old. As naive females may be reluctant to oviposit (van Alphen & Drijver 1982, van Lenteren 1976), each female was first tested for her motivation to lay eggs: the female was placed into a petri dish containing 24 host larvae and observed for 15 minutes. If no oviposition occurred after this time (less than 1/3 of females), the female was discarded from the experiment. Selected females were placed individually into a rearing vial containing 150 host larvae

(respectively n=16 and n=17 for the North and South population of *A. tabida*, and n=15 for each population of *L. heterotoma*) to allow oviposition and thus induce egg maturation (Ellers, Driessen & Sevenster 2000). After eight hours, they were transferred to a vial with food but without hosts. The same procedure was repeated 24h later in a new vial with 150 hosts. After oviposition, females were kept singly in vials with food. At 15d old, they were frozen at -80°C and dissected. The number of mature eggs were measured as previously described. The hosts from the oviposition vials and from the selection of females were reared until the emergence of adults. Emerging parasitoids were collected and counted.

The total number of parasitoids emerging from the selection of the females added to the number of eggs laid in the oviposition vials (taking into account the number of parasitoids emerging and the natural mortality of hosts larvae (unpublished data)) and the number of oocytes in the ovarioles at 15d old was used to estimate potential fecundity.

Dispersal traits

Locomotor activity

The locomotor activity of 7d old mated females was measured during 24h (n=14 for each population of both species). Females were placed individually into a Petri dish (diameter 4 cm) and filmed with a camera Panasonic CCTV. White lights were used during photophase (16L) and red lights during scotophase (8D). The contrast between individuals and a white background was used to detect females and their position was recorded twelve times per minute. The data collected were treated with the software Ethovision (v3.1, Noldus information Technology 1997) which calculates the distance walked (in cm).

Wing loading

Virgin females aged less than 2h were frozen at -80 °C (n=25 for each population of both species). They were first weighted with a microbalance (Sartorius M4±0,001 mg). The area of the left wing was then measured with the numeric image analysis software Pegasus Pro V4 under a binocular (x3.15, Olympus SZ-6045TR) linked to a video camera (JVC KY-F). The wing area enables to calculate the wing loading *i.e.* the ratio of the fresh mass by the wing area, an indicator of the dispersal ability (Starmer & Wolf 1989; Gilchrist *et al.* 2004): individuals with a low wing loading potentially are good dispersers.

Statistical analyses

Climatic data (both mean monthly temperature and mean monthly precipitation from 2004 to 2009) from both localities (North and South) were compared using a two-way ANOVA with repeated measures, using month and locality as factors (GraphPad Prism version 5.01 for Windows, GraphPad Software, San Diego, CA, USA, www.graphpad.com).

To test the effect of the population within each species, linear models were used for the different traits. A linear model was used for egg load at emergence, volume of eggs and wing loading. A generalized linear model with a gamma distribution and power (-1) link function were used for distance walked, metabolism and lipid content. A generalized linear model with a Poisson distribution and a log link function was used for the potential fecundity. A generalized linear model with a Weibull distribution was used for longevity. All models include the size as covariate (fresh mass for wing loading and metabolic rate (Gillooly *et al.* 2001), and tibia length for all other traits (Godfray 1994)), and population as a factor. The models were stepped to the most parsimonious ones using the χ^2 likelihood ratio statistic to eliminate non significant parameters. All tests were conducted with the SAS® software, version 9.1 (SAS Institue, Inc., Cary, North Carolina).

Results

Climatic data

Neither mean monthly temperature (month: $F=182.8$, $d.f = 10$, $P < 0.0001$; locality: $F=0.754$, $d.f. = 1$, $P = 0.4107$) nor mean monthly precipitations (month: $F=0.2.003$, $d.f = 10$, $P = 0.0436$; locality: $F=1.060$, $d.f. = 1$, $P = 0.3333$) were significantly different between the North (Villefranche-sur-Saône) and South (Reventin) (Fig. 1). We can thus assume that very few climatic differences were present between our northern and our southern populations and that climate probably has no strong and direct effect on the difference in life-history traits between our populations.

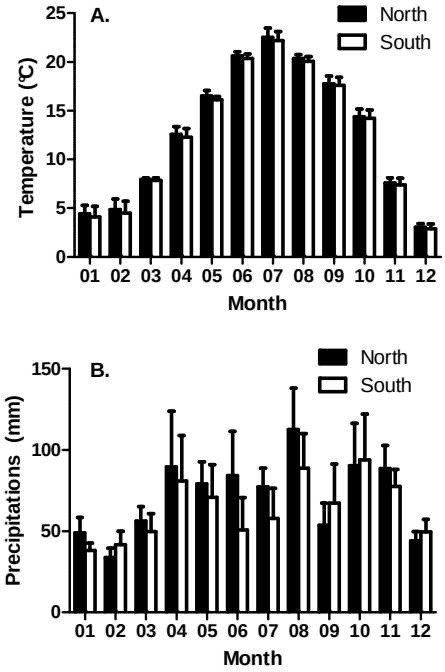

Figure 1. Climate in the North (Villefranche-sur-Saône) and South (Reventin), near the collection sites, from 2004 to 2009. **A.** Mean monthly temperature (°C ± SE) (Month: F = 182.8, d.f. = 10, P < 0.0001; Locality: F = 0.7535, d.f. = 1, P = 0.4107). **B**. Mean monthly precipitations (mm ± SE) (Month: F = 2.003, d.f. = 10, P = 0.0436; Locality: F =1.060, d.f. = 1, P = 0.3333).

Life-history traits

All results are summarized in Table 1.

Table 1. Comparison of northern (N) and southern (S) populations for all traits measured for *A tabida* and *L. heterotoma*. Significant differences between populations within a species are represented in bold.

	Traits	*A. tabida*	*L. heterotoma*
Maintenance	Longevity	**N>S**	N=S
	Metabolic rate	N=S	**N>S**
	Amount of lipids	**N>S**	N=S
Reproduction	Egg load at emergence	**N<S**	N=S
	Potential fecundity	**N<S**	**N<S**
	Volume of eggs	**N<S**	**N<S**
Dispersal	Locomotor activity	**N<S**	N=S
	Wing loading	**N>S**	N=S

Maintenance traits

Asobara tabida females from the North lived longer (Fig. 2; $\chi^2 = 46.7$, d.f. = 1, 103; P < 0.0001) and had more lipids ($\chi^2 = 4.73$, d.f. = 1, 42, P = 0.0297) than females from the South. No difference was observed in longevity (Fig. 2; $\chi^2 = 0.481$; d.f. = 1, 125; P = 0.4882) and amount of lipids ($\chi^2 = 1.56$, d.f. = 1, 46; P = 0.2113) between *L. heterotoma* populations.

The metabolic rate of females was higher in the population from the North than in the population from the South in *L. heterotoma* ($\chi^2 = 4.12$, d.f. = 1, 25, P = 0.0423), but not in *A. tabida*, ($\chi^2 = 0.29$, d.f. = 1, 39 P = 0.5900).

Reproductive traits

The number of mature eggs present in the ovarioles at emergence is higher in the southern population for *A. tabida* ($\chi^2 = 175.3$, d.f. = 1, 48, P < 0.0001) but not for *L. heterotoma* ($\chi^2 = 2.53$, d.f. = 1, 47, P = 0.1117). For both species, females from the South also have bigger eggs at emergence than females from the North (*A. tabida*: $\chi^2 = 6.31$, d.f. = 1, 46, P = 0.0120; *L. heterotoma*: $\chi^2 = 5.09$, d.f. = 1, 46, P = 0.0241).

For both *A. tabida* and *L. heterotoma*, the potential fecundity was lower in the northern population than in the southern one (Fig. 2; *A. tabida*: $\chi^2 = 5.93$, d.f. = 1, 30, P = 0.0149; *L. heterotoma*: $\chi^2 = 10.69$, d.f. = 1, 28, P = 0.0011).

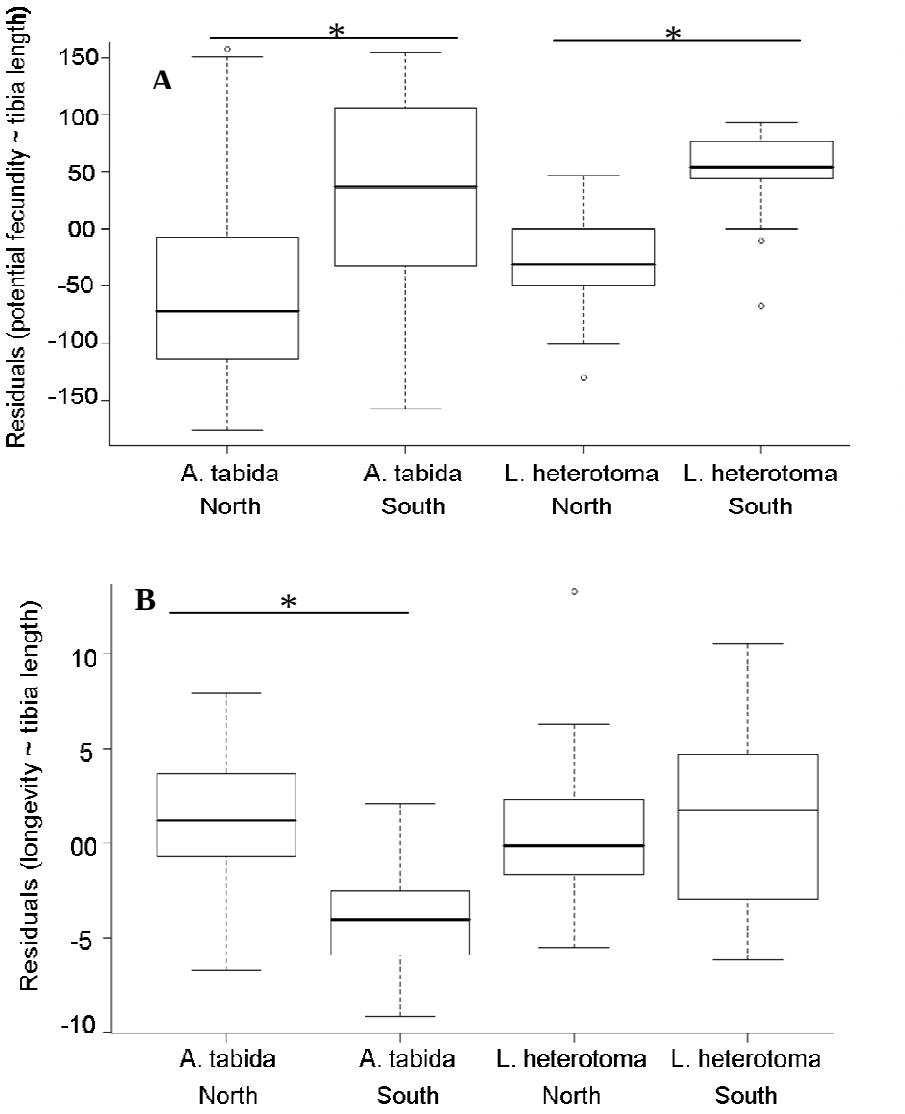

Figure 2. The classical trade-off between reproduction and maintenance in *A. tabida*, but not in *L. heterotoma*: plot of residuals of models comparing traits measured on females from the North and South populations with tibia length as covariate. Asterisk above pairs of bars indicates significant differences between the two populations within the species ($P<0.05$). **A.** Potential fecundity. **B.** Longevity.

Dispersal traits

The distance walked by females was higher for the southern population than for the northern one in *A. tabida* (Fig. 3; $\chi^2 = 8.00$, d.f. = 1, 26, P = 0.0027), but not *L. heterotoma* ($\chi^2 = 3.49$, d.f. = 1, 26, P = 0.0617).

Asobara tabida females from the North have a higher wing loading (0.67 ± 0.08 mg/mm²) and so a lower dispersal ability at emergence than females from the South (0.39 ± 0.07 mg/mm²) (Fig. 3; $\chi^2 = 184.7$, d.f. = 1, 48, P < 0.0001). No significant effect of population on wing loading was detected for *L. heterotoma* (Fig. 3; $\chi^2 = 1.93$, d.f. = 1, 48, P = 0.1645).

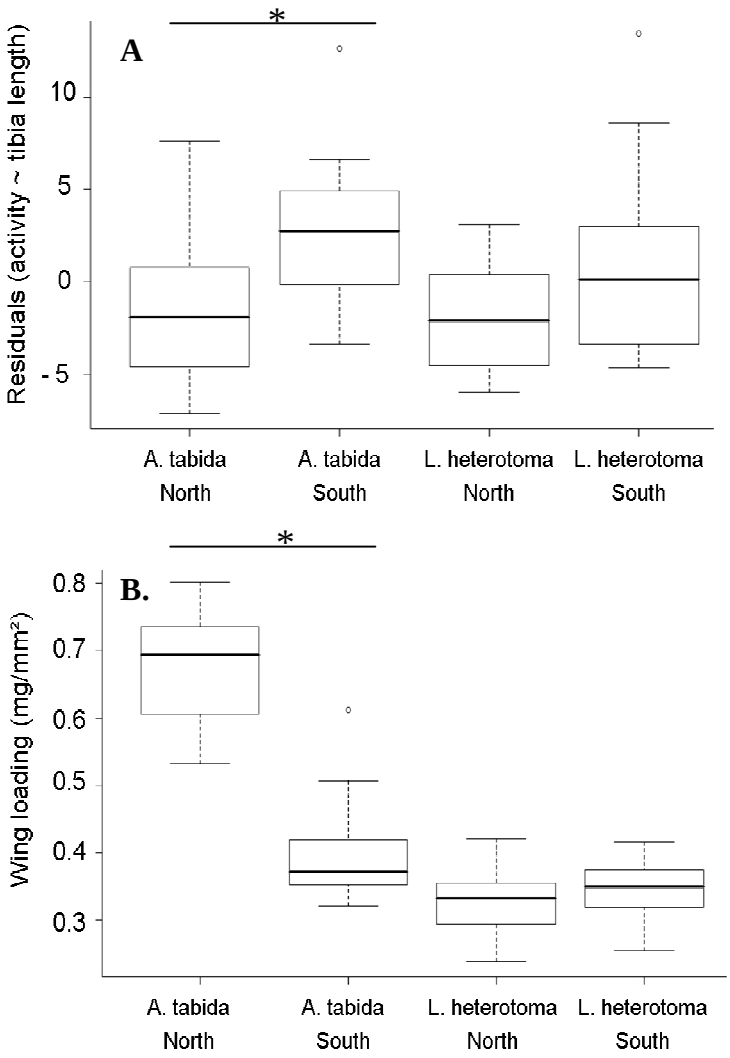

Figure 3. Plot of residuals of models comparing dispersal traits measured on *A. tabida* and *L. heterotoma* females from the North and South populations with tibia length as covariate. Asterisk above pairs of bars indicates significant differences between the two populations within the species (P<0.05). **A.** Locomotor activity. **B.** Wing loading.

Discussion

Potential fecundity is higher in southern populations for both species tested (Table 1). This pattern has already been described for both *A. tabida* (Ellers & van Alphen 1997) and *L. heterotoma* (Ris *et al.* 2004) on larger scales. In these studies, the environmental factors responsible for this difference were identified as habitat, spatial host distribution, climate or host species. In the current study, we have excluded these factors by the proximity of the study sites, the main factor that differs being the history of the parasitoid community: *L. boulardi* has been present for at least 11 years in the southern populations, but for only 2 years in the northern populations. The selection regimes are thus different in these different populations. First, there could be direct competition between *L. boulardi* and the local parasitoids, and as *L. boulardi* is competitively superior (Fleury *et al.* 2000), the resulting mortality would be higher in presence of the competitor. Second, the presence of an additional parasitoid species could also modify the host availability. On 201 second instar *Drosophila* larvae collected from South of France, 181 were parasitized, 44% containing more than one parasitoid larva, whatever the species (Fleury *et al.* 2004). Such a high parasitism rate decreases the proportion of healthy hosts available. The higher mortality and lower host availability in the southern populations (caused by the presence of a competitor) probably explains this higher fecundity, in agreement with Price's (1974) balanced mortality hypothesis.

Females of both species have larger eggs in the South than in the North. This difference goes in the same direction as the potential fecundity, showing a higher investment in reproduction in the southern population. However, this increase in egg volume could also play a role in competition. It has been shown that larger eggs could result in larger neonate larvae (Boivin & Gauvin 2009) and that a higher larval weight could be more advantageous under competition conditions (Parker & Begon 1986).

In *A. tabida*, females from the southern population have a higher potential fecundity and larger eggs, which results in fewer lipids and consequently, a shorter longevity, as expected by the classical trade-off between reproduction and maintenance. In *L. heterotoma*, females from the southern population also have a higher fecundity and larger eggs, but a similar amount of lipids and a similar longevity as females from the northern population. Such an increase in the investment in fecundity should normally be traded-off against longevity. In the current study, as in a previous one (Ris 2003), there is no such trade-off for *L. heterotoma*. However, there is a significant difference in the metabolic rate between the

two *L. heterotoma* populations tested: females from the South have a lower metabolic rate than females from the North. As the metabolic rate is a measure of the speed at which energy is used, a lower metabolic rate could allow for a higher investment in fecundity without any costs in terms of longevity. In contrast, this difference in metabolic rate is lacking in *A. tabida* where females do show the trade-off between fecundity and longevity.

Even though the potential fecundity is higher in southern populations for both *A. tabida* and *L. heterotoma*, the fecundity at emergence is only higher in the southern population for *A. tabida*. A higher fecundity at emergence allows for immediate reproduction following emergence, but prevents the energy needed for it to be invested in maintenance. In *A. tabida*, this higher fecundity at emergence is observed in the population in which females have fewer lipids available at emergence, showing a trade-off in resource allocation during development. As a probable consequence of this lower amount of lipids available at emergence in the southern population, longevity is also reduced in that population (Ellers 1995), as the energy available for maintenance is lower. As female *L. heterotoma* from the southern population show a higher potential fecundity but the same fecundity at emergence as females from the northern population, it suggests that female *L. heterotoma* from the southern population are able to mature more eggs during their adult life with the same initial amount of lipids, probably explained by lipogenesis; as *A. tabida*, *L. heterotoma* is able to synthesize lipids as adult (Visser *et al.* 2010) and is thus not restricted by lipid resources present at emergence. Females *L. heterotoma* thus show a higher plasticity in reproduction as they can adjust their investment in reproduction during their adult life in response to the environment. However, as they have fewer eggs available at emergence, they might experience egg-limitation in the beginning of their reproductive life, in comparison to females with a higher egg load at emergence (Jervis & Ferns 2004).

As expected *A. tabida* females from southern populations have better dispersal abilities. We estimated dispersal abilities using two traits: locomotion activity and wing loading. The locomotion activity is a measure for the general activity of an insect. An insect that is highly active and walks a lot can be expected to have a higher propensity to disperse (Pompanon *et al.* 1995, Fleury *et al.* 2009). Female *A. tabida* from the southern population have higher dispersal abilities through a higher locomotion activity and a lower wing loading. The wing loading is an indication of the physical constraints that an individual experiences because of its wing surface and its weight. In addition, higher dispersal abilities allow exploitation of more hosts in populations with shorter longevity and higher fecundity because higher locomotion activity has been shown to increase oviposition rate in both species (Fleury

et al. 2000). However, a higher dispersal ability is traded-off with longevity in *A. tabida*. There is no such trade-off in *L. heterotoma*: females from the South have the same dispersal ability as females from the North.

In conclusion, changes in the composition of a *Drosophila* parasitoid community due to the arrival of a superior competitor had similar effects on the evolution of key life-history traits in two native parasitoids species. To our knowledge, this is the first study to show the influence of changes in the community on life history traits in parasitoids. The presence of *L. boulardi* as competitor favours *A. tabida* and *L. heterotoma* females with higher fecundity, and *A. tabida* females with higher dispersal ability in southern populations. These differences between populations show how quickly selection can act. In *A. tabida*, the classical trade-off between fecundity and longevity was observed: a higher investment in both reproduction and dispersal at the cost of a lower longevity. In *L. heterotoma*, a lower metabolic rate, and possibly adult lipogenesis allow females to have similar adult lifespan while having the same amount of lipids at emergence, but invest more in reproduction.

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Chapitre 3.

Plasticité phénotypique : adaptations, variations géographiques

Limitation in intrinsic resources affects evolution of metabolic rate in a parasitic wasp

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Key words local adaptations, metabolic rate, lipogenesis, parasitoid, resource distribution.

Abstract

Understanding evolution of metabolism is a central point in the comprehension of evolution of life histories as this physiological trait is closely related to fitness traits such as longevity or growth rate. Metabolism is expected to evolve in response to two main environmental variables: temperature and resources limitation. Investigations of geographical variations of metabolic rate with climate have provided evidence for countergradient variations in the majority of studies, i.e. genotypes for low metabolic rates mainly occur in environments promoting a high metabolic rate (i.e. hot climates). Moreover, a negative correlation between resource limitation and metabolic rate is expected, as a low metabolic rate should be selected in limiting environment to reduce the rate of resources consumption. The main goal of this study was to investigate if differences in limitation in intrinsic resources may have disrupted the expected evolution of metabolic rate in response to climate, i.e. a countergradient variation, in a parasitic wasp.

We compared CO_2 production of four populations of a Drosophila parasitoid, *Leptopilina boulardi*. Two populations originating from a mild and humid climate and unable to synthesize lipids de novo at adult stage were compared with two populations from a desert area where adult lipogenesis occurs. Thus intrinsic resources are less limiting in populations where the metabolic rate should be lower if it has been selected in response to climate.

We observed a cogradient variation in metabolic rate, i.e. high metabolic rate genotypes have been selected in environment promoting a high metabolic rate. We suggest that lipogenesis occurring in the desert area may have allowed the selection of a higher metabolic rate in this environment, as females are less limited in energetic resources than females from mild environment. A high metabolic rate may be adaptive in the desert region as it partly compensates for the long distances that females have to cross to find laying opportunities in

few distant orchards. We suggest that intrinsic resources should be integrated when investigating geographical variations in metabolism as this factor may disrupt evolution.

Introduction

Organisms take up energy from their environment, convert it within their bodies, allocate it to fitness-related traits such as fecundity, longevity or growth and excrete altered forms back into the environment (Brown *et al.* 2004). All these processes define metabolism. Metabolic rate (i.e. the rate of energy uptake, transformation and allocation), generally measured as O_2 consumption or CO_2 production, is thus closely related to the fitness of organisms. For example, an increase in metabolic rate will result in an increase in growth rate (e.g. Metcalfe 1998; Nylin & Gotthard 1998) and a concurrent decrease in development time (Nylin & Gotthard 1998) or longevity (Huey & Stevenson 1979). Metabolic rate is also involved in trade-offs, as described by Crnokrak & Roff (2002) who found a negative correlation between metabolic rate and fecundity in crickets. Thus, understanding the evolution of metabolism is a central element in the comprehension of evolution of life histories.

Metabolism is a biological process that follows physical and chemical laws governing the transformations of energy and materials. Consequently, temperature is the main environmental factor affecting metabolic rate. An increase in metabolic rate when organisms are placed at higher temperatures within a certain range (0 to 40°C generally) has been universally predicted and observed (Clarke & Fraser 2004, Gillooly *et al.* 2001, Nespolo *et al.* 2007). However how metabolism should evolve in response to climate is still highly debated, even if there are clear evidences that local adaptations to climate exist. Two main hypotheses on evolution of metabolism have been described. The most described is the Metabolic Cold Adaptation (MCA) hypothesis, also called Metabolic Compensation hypothesis (Conover & Schultz 1995). The MCA hypothesis assumes that high metabolic rates have been selected in cold environments to compensate for the negative effect of low temperatures on metabolism. This would result in higher metabolic rates in populations or species from cold climates when compared in a common garden with warm-adapted populations or species. Several intraspecific comparisons have argued in favour of MCA in fishes (Álvarez *et al.* 2006, Cano & Nicieza 2006), grasshoppers (Chappell 1983, Massion 1983), *Drosophila* (Berrigan & Partridge 1997), beetles (Strømme *et al.* 1986; Schultz *et al.* 1992) and hymenopteran parasitoids (Le Lann *et al.* in press) but others did not support it (Lardies *et al.* 2004, Lee & Baust 1982, Nylund 1991). Addo-Bediako *et al.* (2002) provided evidence for the MCA hypothesis in a global-scale analysis on interspecific geographical variations of metabolic rate in insects.

The second hypothesis suggests that a low metabolic rate should be selected in hot and dry environments such as deserts (Mueller & Diamond 2001) as it confers resistance to high temperatures and desiccation by reducing exchanges with a stressful environment (Alvarez *et al.* 2006, Massion 1983). Therefore, temperature and humidity are both considered as selective agents in this hypothesis, which received little attention, whereas only temperature is in the MCA hypothesis.

Whichever hypothesis is considered, metabolism is expected to show a countergradient variation when comparing populations from different climates. First defined by Levins (1968) and reviewed later by Conover & Schultz (1995), "countergradient variation occurs when genotypes are distributed in nature such that the genetic and environmental influences on phenotype oppose one another across a gradient". For example, fast-growing genotypes will occur in environments promoting slow growth rate (Arnett & Gotelli 1999, Ligon & Skelly 2009). Countergradient variation opposes to cogradient variation. This term refers to a system where individuals with heritable deviation in a phenotypic character tend to occur in environments that shift the character in the same direction (Conover & Schultz 1995). For example, genotypes for small plant size will be predominant in environments that tend to reduce the size, such as high altitude (Byars *et al.* 2007, Clausen *et al.* 1940). In recent years, investigating these two patterns has received an increasing interest in evolutionary ecology as they partly explain geographic differences in phenotypic variation (Conover *et al.* 2009). Indeed, countergradient variation will result in a decrease in phenotypic variation, as the evolutionary response to the environmental gradient will compensate for the usual phenotypic effect of that gradient (Arendt & Wilson 1999), whereas a cogradient variation will be associated to an increase in phenotypic variation. Few traits show a countergradient variation but this pattern is the general rule for metabolism and associated traits such as growth rate (Pöykkö & Tammaru 2010, for a review, see Conover *et al.* 2009). To our knowledge, cogradient variation has never been described in empirical or modelling studies for these physiological traits. Thus comparing populations from different climates, low metabolic rate genotypes should be observed in environments promoting a high metabolic rate (i.e. hot climate) if this trait has been selected in response to climatic variables.

A second important ecological factor in evolution of metabolic rate is the limitation in resources. Indeed, the higher the metabolic rate, the faster resources are consumed and allocated to fitness-related traits. Consequently, a lower metabolic rate is expected in marginal environments where resources are limiting or unpredictable, in order to reduce the rate of resources consumption (Hoffmann & Parsons 1997, Mueller & Diamond 2001). Therefore

results may be different than a countergradient variation in metabolic rate if geographic variations in climate correlate with variations in resources availability. The main goal of this study was precisely to investigate if differences in resources limitation may have affected the expected evolution of metabolic rate in response to climate, i.e. a countergradient variation, in an hymenopteran parasitoid. However, we did not consider environmental but intrinsic resources as potential disruptor of countergradient variation.

Lipids represent the main energetic resource allocated to survival, reproduction and dispersion in parasitoids (Ellers & van Alphen, 1997, Ellers *et al.* 1998, Rivero & Casas, 1999). These organisms were thought to be unable to synthesize lipids during adult life (for a review, see Visser *et al.* 2010) but Moiroux *et al.* (2010) found intraspecific variations in the lipogenesis ability between populations of a *Drosophila* parasitoid, *Leptopilina boulardi*, originating from different climates. Females from an Iranian desert area were able to synthesize lipids during adult life whereas populations from the mild and humid Iranian coast of the Caspian Sea did not. Thus the limitation in intrinsic resources is different between populations from different climates and ability to produce additional lipids during adult life may have affected the evolution of metabolic rate. We compared the CO_2 production of females from these Iranian populations and investigated if a countergradient variation occurs (i.e. a lower metabolic rate in populations from the hottest areas) as suggested by other studies on evolution of metabolism or if differences in lipogenesis ability have affected evolution of metabolic rate.

Material & Methods

Rearing

Four populations of *Leptopilina boulardi*, a solitary endoparasitoid that mainly attacks *D. melanogaster* and *D. simulans* larvae were collected in July 2006 in Iran in a mild region and a desert region, using twelve banana bait traps per site. Each open trap (i. e. a plastic container with a 3 cm diameter hole covered with a mesh with 2 mm openings) was colonised by five to twenty females and several hundreds of offspring were produced in each of them. From the offspring thirty females per population were taken to set up lab cultures. Two populations - Chalus and Seyakhal – originated from a humid and mild environment, in the coastal plain of the Caspian Sea. Dorcheh and Zamankhan strains originated from a hot and very dry area

close to Esfahan, although the location of our traps was in the valley of a snow-fed river that keeps water throughout most summers. Climatic data are presented in table 1.

Drosophila melanogaster used as hosts in the cultures originating from strains collected in the Netherlands in 1960. Parasitoids and drosophila were reared at 25 ± 1 °C, the optimal rearing temperature for *L. boulardi*, 0 ± 10 % RH and 16L: 8D photoperiod.

Table 1. Climatic data recorded for the four sampled areas, from 1977 to 2005 for Chalus, 1955 to 2005 for Seyakhal and Zamankhan, 1951 to 2005 for Dorcheh (Source: Islamic Republic Of Iran Meteorological Organization). We considered average of each parameter measured every day from April to September, which is the period of activity of *Leptopilina boulardi*. Thermal amplitude was calculated as the mean of the differences between maximum and minimum air temperature per month.

Sampling areas	Temperature (℃)	Thermal amplitude (℃)	Relative humidity (%)	Number of rainy days	Precipitation amount (mm)
Chalus	26.3	7.6	80	43.3	248
Seyakhal	26.4	9.8	79	56.7	433
Dorcheh	31.6	15.6	29.7	14.2	30.9
Zamankhan	28.6	19.2	36	13.8	55

For the experiment, *L. boulardi* females oviposited in separate jars for 24h at 25°C in 150 second instar *D. melanogaster* larvae laid in a two hours period (similar size for every host) in a baker's yeast suspension. The parasitized larvae were then separated in four groups which were placed at 20, 22.5, 25 or 27.5°C. Metabolic rate and amount of lipids were measured at four temperatures as different reaction norms may have been selected in different climates. When emerging, wasps were separated in two groups. The first one was used for measures of metabolic rate and the second one was used for measures of lipids.

No individuals from Dorcheh emerged at 20°C thus no data are available at this temperature for this population.

Metabolic rate

We used flow-through respirometry to measure metabolism of emerging female wasps. Fifteen to twenty samples per temperature were tested for each population. To detect a strong signal, twenty individuals were pooled per sample. Their fresh mass was measured with a microbalance Sartorius, samples of twenty 1-d old individuals were placed in climate room at rearing temperature in small cylindrical chambers and their CO_2 production was measured

with an infra-red CO_2 analyser (LI-COR LI6251). A Sierra mass flow controller maintained constant flow rates of dry, CO_2-free air. Air was drawn off from the environment and CO_2 and water were scrubbed with a Drierite-Ascarite column. Four records of five minutes for each sample were performed with a lag of 90 minutes between two recordings, and were automatically transformed by a program recorded in DATACAN software (Sable Systems, Las Vegas), to convert the measure from ppm to µL-CO_2 per hour, taking into account the flow rate. The first recording allowed individuals to get used to their new environment and was not considered for analyses. We considered the average of the three last recordings as a measure of metabolic rate for each sample. Assay were performed during the photophase (10:00 am to 04:00 pm) and scotophase (6:00 pm to 12:00 pm) to cover different periods as differences in daily activity occur among the four populations (Moiroux *et al.* 2010).

Lipid analysis

Amount of lipids was investigated at three ages to detect lipogenesis during adult life. Adult parasitoids used for measures of lipid quantity were reared at the same larval development temperature, 12L: 12D in glass jars on an Agar-Nipagine substrate and fed with diluted acacia honey distributed *ad libitum*. We measured the lipid content of 20 females per age and temperature for each population, using a colorimetric analysis protocol developed for parasitoids by Giron *et al.* (2002) and adapted from van Handel (1985). Twenty 1-d, 5-d and 10-d old females were placed in Eppendorf tubes, killed in liquid nitrogen and conserved at –80°C. After defrosting, left hind tibia length was measured (±0.01mm) with the numeric image analysis software Pegasus Pro V4 under a binocular (Olympus SZ-6045TR) linked to a camera video (JVC KY-F). Then ovaries were both removed, as we were interested in stored lipids, and wasps were individually placed in Eppendorf tubes in 50 µL of Ringer solution and crushed with a plastic pestle in 300 µL of methanol. Tubes were centrifuged for 15 minutes at 4°C and 1400 rpm. 150 µL of chloroform and 60 µL of 2% sodium sulphate solution were then added to samples and tubes were vortexed and stored at 4°C during one night. The next day, tubes were centrifuged for 15 minutes at 1400 rpm. 300 µL of supernatant per sample were transferred to other tubes that were put on an aluminium heat block at 90°C until total evaporation. 40 µL of 98% Sulfuric Acid were then added, tubes were heated again at 90°C for two minutes and cooled down on ice. 960 µL of Vanillin Reagent were added and tubes were vortexed and left for 15 minutes at ambient temperature. The absorbance was read at

525 nm with a spectrophotometer (VersaMAX). The calibration curve we used to transform absorbance into concentrations was made with standard sunflower oil.

Statistical analysis

Metabolic rate increases with body size (Gillooly *et al.* 2001). Thus we performed ANCOVA with sampling site as fixed factor and fresh mass as covariate to test differences in metabolic rate corrected for body size. Metabolic rate with and without light were tested in separate models.

We also used analysis of covariance ANCOVA to analyze the age effect on amount of lipids with tibia length as covariate for each population and each temperature, to detect lipogenesis.

We performed Generalized Linear Models using the global dataset to test for correlations between metabolic rate and climatic factors of origin: air temperature, thermal amplitude, number of rainy days and precipitation amount. Climatic factors were included in the base model and removed one by one to obtain the minimum adequate model. The selection was done on the basis of the AIC criterion (Akaike Criterion, Akaike 1974). P-values reported are those of the last model that contains the variables.

All analyses were carried out using R software version 2.8.1 (R Development Core Team, 2008).

Results

Metabolic rate

Sampling site had a significant effect on metabolic rate. Populations from the desert area had generally a higher metabolic rate than those from the mild and humid climate at every temperature, as well as at the different times in the photoperiod (Figure 1). Dorcheh and Zamankhan had a higher metabolic rate than Seyakhal and Chalus with fresh mass as covariate at 22.5°C (Light: df = 3, 72, F = 4.007, p = 0.012; No light: df = 3, 72, F = 3.56, p = 0.02), 25°C (Light: df = 3, 72, F = 3.037, p = 0.01; No light: df = 3, 72, F = 3.56, p = 0.02) and 27.5°C (Light, df = 3, 62, F = 2.91,p = 0.036; No light, df = 3, 62, F = 4.09, p = 0.019). Zamankhan had a higher metabolic rate than Seyakhal and Chalus at 20°C with light (df = 2,

52, F = 2.72, p = 0.045) but no difference occurred in darkness (df = 2, 52, F = 0.85, p = 0.43).

In every population, we observed a significant effect of rearing temperature on metabolic rate and there were no differences between populations in the shape of metabolic rate reaction norms (Figure 1). Metabolic rate with light increased with temperature between 20°C, 22.5°C and 25°C and decreased between 25°C and 27.5°C in every population (Chalus: df = 3, 68, F= 5.105, p = 0.015, Seyakhal: df = 3, 74, F = 3.65, p = 0.018, Dorcheh: df = 2, 50, F = 2.43, p = 0.044, Zamankhan: df = 3, 71, F = 3.45, p = 0.027). The same pattern was observed in darkness (Chalus: df = 3, 68, F = 1.92, p = 0.051, Seyakhal: df = 3, 74, F = 2.93, p = 0.035, Dorcheh: df = 2, 50, F = 3.912, p = 0.028, Zamankhan: df = 3, 71, F = 0.442, p = 0.008) except that no difference occurred between 20°C and 22.5°C for Chalus and Seyakhal population.

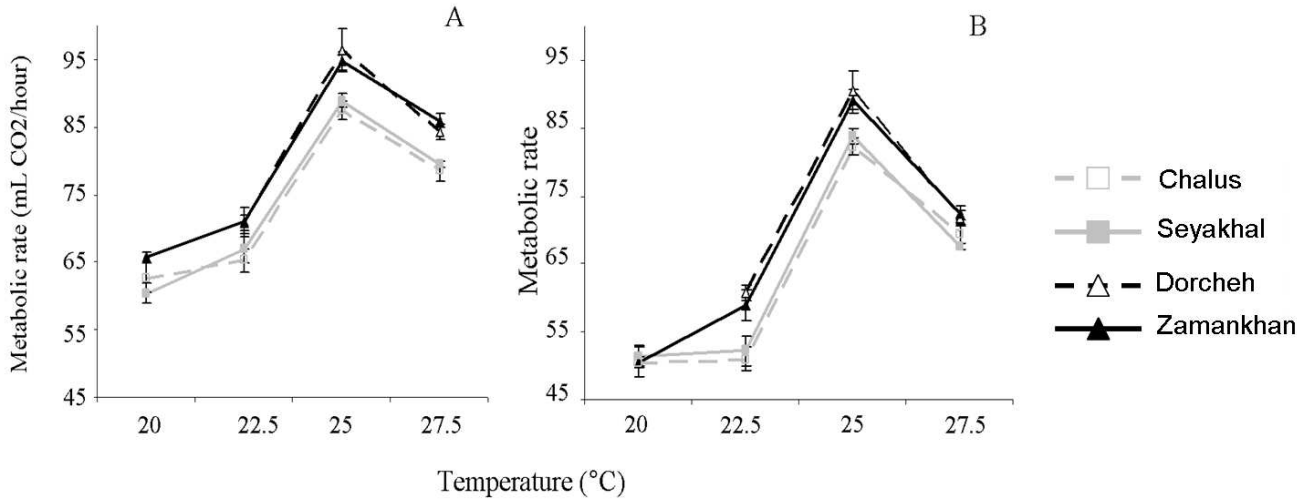

Figure 1. Metabolic rate during photophase (A) and scotophase (B) measured at four temperatures for the four populations. Error bars: ± s.e.. No individual from Dorcheh emerged at 20°C.

We did not observe any significant correlation between metabolic rate and climatic factors of origin (GLM, F = 1.13, p = 0.14) although precipitation amount was closest to be significant (GLM, F = 1.84, p = 0.087). Geographic variations in metabolic rate could thus not be clearly correlated to regional climatic factors of origin.

Lipid analysis

There was no evidence that lipogenesis occur in females from the mild and humid environment but did in those from the desert area indicate (figure 2). We observed a significant decrease in lipid quantity between emergence, five days and ten days in Seyakhal and Chalus females reared at 20°C (Seyakhal: df = 5, 56, F = 8.13, p < 0.001; Chalus: df = 5, 56, F = 19.03, p < 0.001), 22.5°C (Seyakhal: df = 5, 56, F = 14.12, p = 0.002; Chalus: df = 5, 56, F = 9.87, p <0.001), 25°C (Seyakhal: df = 5, 56, F = 27.34, p < 0.0001; Chalus: df = 5, 54, F = 23.33, p <0.0001) and 27.5°C (Seyakhal: df = 5, 46, F = 3.052, p = 0.047; Chalus: df = 5, 44, F = 6.14, p = 0.017). At 22.5°C and 25°C, we observed a significant increase in lipid quantity in females from Zamankhan (22.5°C: df = 5, 56, F = 14.03, p < 0.001; 25°C: df = 5, 56, F = 7.25, p < 0.01) and Dorcheh (22.5°C: df = 5, 54, F = 4.11, p = 0.023; 25°C: df = 5, 56, F = 3.977, p = 0.0377) between emergence and five days but no difference was observed between five days and ten days. No significant difference was observed at 27.5°C in Dorcheh (df = 5, 52, F = 3.056, p = 0.094) and Zamankhan females (df = 5, 54, F = 3.62, p =0.01) between emergence, five days and ten days nor at 20°C in Zamankhan females (F = 0.149, p = 0.923). There is thus no clear evidence of lipogenesis at these two temperatures but no decrease in lipid content suggests that it may also occur at these temperatures.

Females from Chalus and Seyakhal emerged with more lipids than females from the desert at every temperature (df = 7, 74, F = 4.89, p = 0.004). The opposite pattern occurred at five (df = 7, 72, F = 5. 21, p = 0.01) and ten days (df = 70, F = 6.80, p = 0.014).

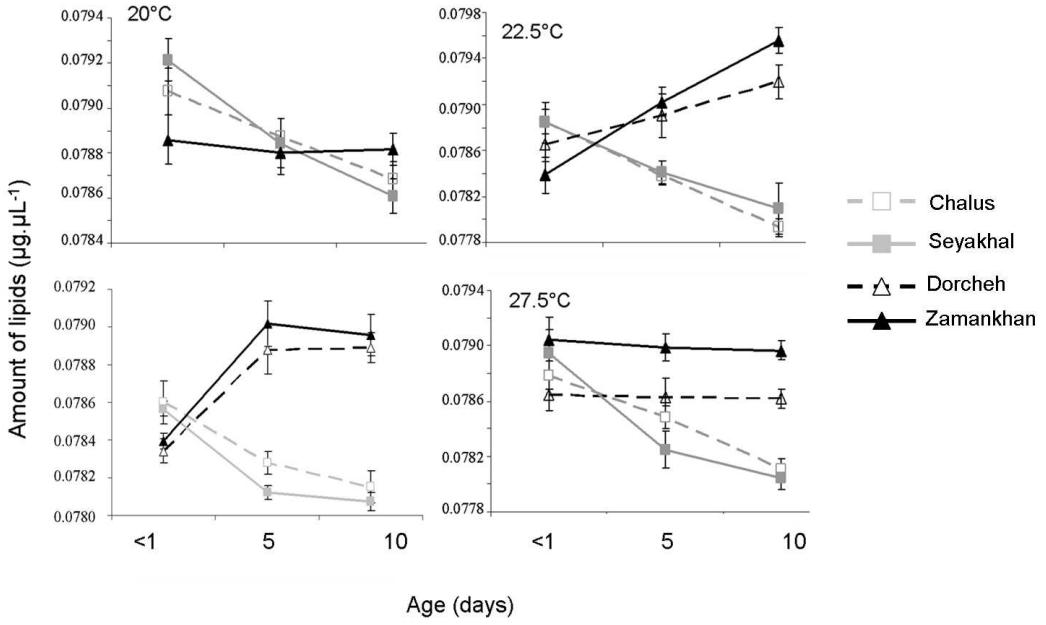

Figure 2. Temporal variations in the amount of lipid, at four populations. Error bars: ± s.e. No individual from Dorcheh emerged at 20°C.

Discussion

We observed that females living in an environment where temperature promotes a high metabolic rate (i.e. the hottest area) had the highest metabolic rate, independently of rearing temperature or presence/absence of light. These results suggest that a cogradient variation in metabolic rate occurs (Conover & Schultz 1995). To our knowledge, this is the first time that cogradient variation in this trait is observed in terrestrial arthropods. Our results are in contradiction with the great majority of studies on the evolution of metabolism in response to climate where countergradient variations have been observed, i.e. populations/species from cold climate have a higher metabolic rate than the ones from hot climate (e.g. Addo-Bediako *et al.* 2002, Berrigan & Partridge 1997). This particular geographic pattern involves that a strong phenotypic variation occurs between our sampling areas whereas a low phenotypic variation is generally observed when comparing populations from different climates. Authors suggest that high metabolic rates have been selected in cold environments to compensate for the negative effect of low temperatures on metabolism, or that low metabolic rates have been selected in hot and dry climates to limit effects of desiccation and stressful temperature (i.e. a lower metabolic rate in desert area, Massion 1983). Therefore our opposite results to these

studies suggest that geographic variations of metabolic rate have not been selected in response to climate of origin.

Moreover, if environmental resources had selected for the metabolic rate, we should have observed the lowest in desert females as selection for reduced metabolic rate is expected to be strong in marginal environments where resources are limiting or unpredictable (Hoffmann & Parsons 1997, Mueller & Diamond 2001), such as our desert area. Thus geographic variations in environmental resources are unlikely to explain cogradient variation.

Our results show that lipogenesis occurs in populations from the desert area and is unlikely to occur in populations from the mild and humid area, as found in a previous study at 25°C (Moiroux *et al.* 2010). When feeding on carbohydrate sources such as nectar, desert females produce lipids *de novo* whereas females from the mild climate do not. The consequence of these differences is that limitation in intrinsic resources is stronger in females from the mild climate, even if they emerge with more lipids than desert females as observed in this study. In the desert area, parasitic wasps have to cross large distances because laying opportunities are concentrated in a few distant orchards distributed along a river. In a previous study, Moiroux *et al.* (2010) suggested that lipogenesis may have been selected in populations from the desert area as a large amount of lipids is required to fly over large distances (Ellers *et al.* 1998). The present study indicates other evolutionary advantages for lipogenesis. The decrease in resource limitation (i.e. lipogenesis ability) may have allowed the selection of a higher metabolic rate in the desert. In this environment, a high metabolic rate may be adaptive as it allows wasps to use resources faster and to be more active (Brett & Groves 1979, Cano & Nicieza 2006), without living shorter (Moiroux *et al.* 2010). That increased locomotor activity would compensate for the long distances between patches of hosts and result in a higher number of hosts encountered, and therefore in a higher progeny. Thus, differences in host distribution and lipogenesis may explain the cogradient variation that we observed. Moreover, lipids synthesized during adult life may limit desiccation (Hadley, 1994) and partially compensate for the additional water loss due to a higher metabolic rate (Clarke 1993, Massion 1983).

Metabolic rate increased with temperature between 20°C and 22.5°C as found in all studies on temperature-dependence of metabolism in insects and a majority of ectotherms. However we observed a decrease between 25°C and 27.5°C suggesting that this last temperature is physiologically stressful and may negatively affect metabolism and activity of organisms. We found in a previous study (Moiroux *et al.* 2010) that desert females were more active during the night. In this paper, we suggested that this shift to a nocturnal activity was

adaptive as that behaviour would limit exposure to extreme diurnal temperatures. Results on metabolic rate at 27.5°C tend to confirm that avoidance of high temperatures may be adaptive as these temperatures may be physiologically stressful.

Even if we observed a higher metabolic rate in desert females than in mild females, similar metabolic rate reaction norms were found in the four populations. The elevation was different but the slopes were very close. Our results suggest that no selection on reaction norms of metabolic rate nor on strength of phenotypic plasticity occurred. To our knowledge, inter-or intraspecific variations in metabolic rate reaction norms have never been investigated. Metabolism obeys to strict physical and chemical principles governing the transformations of energy and materials, most relevant being laws of mass and energy balance and thermodynamics (Brown *et al.* 2004). It is likely that evolution can only select for the mean values of the reaction norm and not the slope as that would probably involve selecting for changes in these laws.

Conclusion

Evolution of metabolism, a central element in evolution of life history traits, has always been considered as the result of environmental factors, particularly temperature and environmental resources limitation or unpredictability. Our study argues that host distribution may select for metabolic rate. We suggest that differences in physiological constraints between populations may have affected this evolution and should be integrated when investigating geographic variations of metabolism.

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Geographical variations in the level of phenotypic plasticity: Does reproductive strategy or environmental variation explain it?

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Abstract

Phenotypic plasticity, the ability of a given genotype to produce different phenotypes when exposed to different environments, is thought to be adaptive. The level of this phenotypic plasticity, which is represented by the slope of a reaction norm, shows heritable genetic variation, and is thus able to evolve. Environmental variation is known to affect it: a flat reaction norm is observed in variable environments, where fitness-related life history traits should be buffered against important variations. Life histories may also affect this level of plasticity, such as egg maturation strategy in insects, but this assumption has never been investigated. Two strategies have been described in parasitic wasps: proovigeny, where females emerge with all their eggs mature and are not able to produce additional eggs during adult life, and synovigeny, where females can produce additional eggs during adult life. A flat reaction norm may thus be expected in proovigenic wasps as their fecundity should be buffered against environmental variations.

In this study, we investigated the relative role of egg maturation strategy and environmental variation in the selection of the level of phenotypic plasticity of fitness-related traits in parasitic wasps. To address this question, we compared thermal reaction norms of egg load, egg size and amount of nutrients of proovigenic and synovigenic populations/species from two different climates.

In populations/species from the stable climate, we observed a strong decrease in egg load and an increase in egg size with an increase in temperature. This reaction norm may be an adaptation to the effect of temperature on the size of hosts. As smaller hosts occur at higher temperature, parasitic wasps may lay bigger eggs to compensate for the lower amount of nutrients provided by the host. Similar reaction norms of egg load, egg size and amount of nutrients were observed in proovigenic and synovigenic species from the stable climate, whereas they were flatter in populations from the variable environment. These results indicate that the level of phenotypic plasticity is primarily affected by environmental variation and not intrinsic characteristics such as egg maturation strategy in parasitoids.

Introduction

The phenotype of every organism is determined by its genotype, its environment and the interaction between both. A given genotype can produce different phenotypes when exposed to different environments (Johannsen 1911). This ability defines phenotypic plasticity (Bradshaw 1965), a major immediate response of organisms to the environment which is thought to be adaptive (Lyytinen *et al.* 2004, Pigliucci 2005). Many environmental factors can interact with the genotype and affect the phenotype, such as quantity of food (Woltereck 1909, Chapman *et al.* 2010), predation risk (Krueger & Dodson 1981, Petrin *et al.* 2010), conspecific density (Leips *et al.* 2009) or wind (Garcia-Verdugo *et al.* 2009), but temperature has been by far the most investigated (e.g. Azevedo *et al.* 1996, Colinet *et al.* 2007, Le Lann *et al.* 2010). The phenotypic plasticity of a trait can be characterised by its reaction norm, i.e. a function, with the value of the environmental variable as argument and the value of the trait as function value (de Jong 1990).

The level of phenotypic plasticity, which can be measured by the slope of the reaction norm (de Jong 1990), often shows heritable genetic variation (Huey & Kingsolver 1989) and thus can evolve. Environmental variation is known to affect the level of plasticity: a low level of plasticity is expected in traits closely related to fitness, such as fecundity, in variable environments (Wagner *et al.* 1997). This canalisation will buffer traits against environmental variation (Richards *et al.* 2006), limiting a strong decrease in fitness when the environment is away from the optimum. For example, the rate of developmental, a fitness trait that varies with temperature, is more canalised in *Drosophila serrata* populations from a temperate climate (i.e. a variable climate) than in those from a tropical climate (i.e. a stable climate) (Liefting *et al.* 2009). Other environmental factors can be correlated to different levels of phenotypic plasticity, such as the differences in prey size between Australian mainland and islands populations of tiger snakes that may have selected for higher level of plasticity in jaw size in island populations where prey are bigger (Aubret *et al.* 2004).

Environmental factors can affect reaction norms but we may also expect that different life histories may result in different levels of phenotypic plasticity. Wimberger (1991) proposed for example that the age of first-feeding in cichlid fishes, which depend on the mode of parental care, may affect the level of plasticity in jaw morphology. However, importance of intrinsic characteristics, such as reproductive strategies, on the level of plasticity has been rarely investigated. Furthermore the few studies where this question was addressed failed to

provide support for this hypothesis (e.g. Wimberger 1991). In this study, we tested the relative role of life histories in parasitic wasps, particularly egg maturation strategies, and environmental variation in the selection of thermal reaction norms of fitness-related traits. We mainly focused on egg load but we also investigated egg size, as there is a negative correlation between temperature and these two traits (Fischer et al. 2003). Moreover, we tested the reaction norms of longevity and the amount of stored nutrients.

In parasitoids, there are two strategies of egg maturation (Flanders 1950). The first one is proovigeny, where females emerge with all their eggs mature and they can not produce others eggs during adult life. The second strategy is called synovigeny, where females emerge with only a part of their eggs mature and can develop otehrs during adult life. These species can adjust their allocation of resources to reproduction according to the laying opportunities (Jervis *et al.* 2001). They are, thus, considered more plastic as they can allocate their resources to reproduction or maintenance during adult life, whereas the majority of resources have been invested in reproduction before metamorphosis in proovigenic species.

We compared thermal reaction norms of four populations of a *Drosophila* parasitoid, *Leptopilina boulardi*: two were synovigenic and originating from a desert area, a variable environment, and two were proovigenic and originating from a mild and humid climate, a more stable environment (Moiroux *et al.* 2010). To separate effects of life histories and environment on level of phenotypic plasticity, we also investigated reaction norms of a *L. heterotoma* population, a synovigenic species, sampled in the same area as proovigenic populations of *L. boulardi*. We hypothesized that (1) if reaction norms have been selected in response to the egg maturation strategy, fecundity would be less plastic in proovigenic populations than in synovigenic ones. We thus expect that reaction norms of egg load would be flatter in proovigenic *L. boulardi* populations than in synovigenic populations of *L. boulardi* and *L. heterotoma*. (2) if environmental variation has selected for reaction norms, no difference will occur between the two species from the same climate and the reaction norms in egg load and egg size will be flatter in populations from the desert area.

Material & Methods

Source of laboratory samples

Four populations of *Leptopilina boulardi* and one population of *L. heterotoma*, two solitary endoparasitoids that mainly attack *D. melanogaster and D. simulans*, were collected in July

2006 in Iran as described in Moiroux *et al.* (2010). We collected two populations of *L. boulardi* - Dorcheh (32°37'00''N, 51°32'59''E) and Zamankhan - in a hot and very dry area close to Esfahan. Two other populations of *L. boulardi*, Chalus (36°39'17''N, 51°25'18''E) and Seyakhal (37°19'08''N, 49°22'11''E), were sampled in the coastal plain of the Caspian Sea, in a mild and humid climate. The population of *L. heterotoma* studied was also sampled in Chalus int the same period. The two *L. boulardi* populations from the Caspian Sea were described as proovigenic and unable to synthesise lipids during adult life, as the great majority of parasitoid species (Visser & Ellers 2008), whereas synovigeny and lipogenesis ability occur in desert populations (Moiroux *et al.* 2010). *L. heterotoma* is known to be synovigenic and able to synthesise lipids during adult life (Visser *et al.* 2010, Moiroux, unpublished data).

Cultures

Drosophila melanogaster used as hosts in the cultures originating from strains collected in the Netherlands in 1960. *L. boulardi* females oviposited in separate jars for 24h at 25°C in 150 second instar *D. melanogaster* larvae in a baker's yeast suspension. The parasitized larvae were then separated in four groups and placed at 20, 22.5, 25 or 27.5°C. When emerging, wasps were separated in two groups per temperature. The first one was used for measures of morphometric traits, egg load, amount of nutrients and the second one was used for longevity. No individuals from Dorcheh emerged at 20°C thus no data are available at this temperature for this population. Diapause may occur at this temperature for this population from the hottest climate. Pupae were transferred from 20°C to 25°C but no wasp emerged.

Life history traits

Morphometric traits, number and volume of eggs, amount of lipids and sugars were measured on 20 emerging females per population per temperature. Females were placed in Eppendorf tubes, frozen in liquid nitrogen and conserved at -80°C.

Reproduction. After defrosting, fresh mass of females was determined with a microbalance (Mettler Toledo XP2U ± 0.1µg) while left hind tibia was measured (±0.01mm) with the numeric image analysis software Pegasus Pro V4 under a binocular (Olympus SZ6045TR)

linked to a camera video (JVC KY-F). Females were then placed in 25 µL of Ringer's solution on a microscope slide and dissected under a binocular (×40, Olympus SZX9). We removed eggs from both ovaries and counted them, while the rest of the female's body was washed with 25 µL of Ringer's solution and transferred in Eppendorf tube for later nutrient analysis. After counting, eggs were placed under a microscope (×4, Olympus BH2) and photographed (Olympus Camedia C3040). Length (L) and width (w) of 30 mature eggs per female were measured with the numeric image analysis software AnalySIS to calculate eggs volume (taken as a prolate spheroid volume: $V=4/3\pi Lw^2$).

Nutrients analyses. After eggs had been removed from both ovaries, we measured the amount of lipids and sugars for each female, using a colorimetric analysis protocol developed for parasitoids by Giron *et al.* (2002). Wasps placed in individuals Eppendorf tubes (tubes A) with 50 µL of Ringer solution were crushed with a plastic pestle in 300 µL of methanol. Tubes were centrifuged for 15 minutes at 4°C and 1400 rpm. We then added 150 µL of chloroform and 60 µL of 2% sodium sulphate solution and tubes were vortexed and stored at 4°C during one night. The next day, tubes were again centrifuged for 15 minutes at 1400 rpm.
For the lipid assay, 300 µl of the supernatant from each female were heated on an aluminium heat block at 90°C until total evaporation. 40 µl of 98% sulfuric acid were then added. The samples were heated at 90°C for 2min and cooled on ice for five minutes. 960 µL of Vanillin Reagent were added and samples were vortexed and left for 15 minutes at ambient temperature. The absorbance was read at 525 nm with a spectrophotometer (VersaMAX). The calibration curve we used for lipids was made with standard sunflower oil.
For the sugar analysis, we transferred 150 µL of the supernatant from samples (Tube A) to other tubes which were heated at 90°C on aluminium block and removed before complete evaporation. We then added 1 ml of anthrone reagent in tubes, heated them for 15 min at 90°C and cooled them down on ice for 5 min. The absorbance was read at 625 nm. We used glucose to establish the calibration curve for sugars.

Longevity. This parameter was measured in the second group of virgin wasps (n=25 females per population per temperature). Females from each population were placed at developmental temperature on an Agar-Nipagine substrate and were fed with diluted acacia honey distributed *ad libitum*. The substrate was renewed every week. Dead individuals were counted and removed twice a day, each morning and evening.

Statistical analysis

We compared egg load, egg volume and amount of lipids with an ANCOVA, as these variables were normally distributed. The site (mild vs desert area) and egg maturation strategy (proovigenic vs synovigenic) were included as explanative variables and tibia length was included as covariate. A model was tested between populations for each temperature and between temperatures for each population. The selection was done on the basis of the AIC criterion (Akaike Criterion, Akaike 1974). P-values reported are those of the last model that contains the variables.

Amount of sugars were compared with generalized additive model assuming a binomial distribution of data. The sampling site and egg maturation strategy were included as explanative variables and tibia length as covariate. A model was tested between populations for each temperature and between temperatures for each population.

The survival package provided by R software was used to test differences in longevity, using a Weibull distribution, with sampling site and egg maturation strategy as explanative variables. Rearing temperatures were tested separately.

The mean values of each trait were calculated per population per temperature, to determine the slope of reaction norms between 22.5°C-25°C and 25°C-27.5°C. We performed ANOVA to compare the slope of reaction norms with sampling site and egg maturation strategy as explanative variables. As no individuals from Dorcheh emerged at 20°C, we could not test statistically for the slope between 20°C-22.5°C as we had only one synovigenic population from the desert area.

All analyses were carried out using R software version 2.8.1 (R Development Core Team, 2008).

Results

Reproduction

We observed a significant effect of sampling site and not egg maturation strategy on the slopes of egg load reaction norms between 20°C and 22.5°C, between 22.5°C and 25°C (F =

87.99.16, df = 2, p = 0.011) and 25°C-27.5°C (F = 38.44, df = 2, p = 0.014). Egg load reaction norms decreased with an increase in temperature in species/populations from the mild climate whereas they were flat in *L. boulardi* from the desert area (Figure 1).

The interaction sampling site*egg maturation strategy had a significant effect on initial egg load at 20°C (F = 36.44, df = 2, 56; p < 0.001) and 22.5°C (F = 19.71, df = 3, 76; p < 0.001). No differences between populations were observed at 25°C (F = 3.76, df = 3, 76, p = 0.12) and climate of origin as only variable explained differences between populations at 27.5°C (F = 36.44, df = 3, 76; p < 0.001). Proovigenic *L. boulardi* populations emerged with more eggs than synovigenic populations of the same species at 20°C and 22.5°C whereas the opposite result was observed at 27.5°C. *L. heterotoma* exhibited an intermediate egg load between these populations at 20°C and 22.5°C and a similar egg load than proovigenic populations at 27.5°C

The slopes of egg volume reaction norm differed between females from different sampling sites between 20°C-22.5°C, 22.5°-25°C (F = 15.0, df = 2, p = 0.046) and 25°C-27.5°C (F = 21.5, df = 2, p = 0.022). Females from the desert area had a flatter reaction norm than populations from mild climates. We observed a low but significant increase in egg size from 20°C to 25°C in populations from the mild climate, and a decrease at 27.5°C. Temperature did not affect egg volume in desert populations (F = 3.18, df = 3, 36, p = 0.21).

Wasps from the mild climate emerged with smaller eggs than desert wasps at 20°C (F = 41.21, df = 3, 67, p = 0.043) and 27.5°C (F = 66.17, df = 3, 76, p = 0.037). No difference between populations was detected at 22.5°C (F = 8.02, df = 3, 62, p = 0.27) and 25°C (F = 14.12, df = 3, 76, p = 0.40).

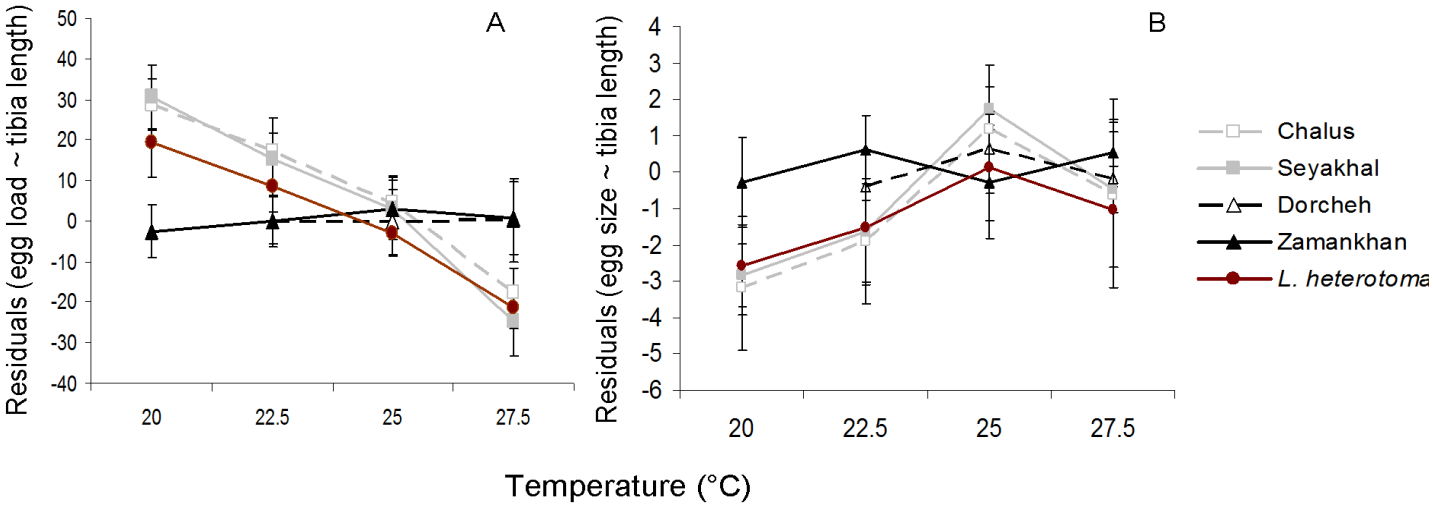

Figure 1. Residuals of models comparing (A) egg load and (B) egg size at emergence with tibia length as covariate in response to four developmental temperatures. One model was tested per population. The higher the residuals, the more females had eggs for a same body size. Errror bars: ± SE.

Nutrients

Egg maturation strategy had an effect on the slope of reaction norms for amount of lipids. Synovigenic populations/species had flatter reaction norms than proovigenic populations between 20°C-22.5°C, 22.5°C-25°C ($F = 81.8$, $df = 2$, $p = 0.009$), 25°C-27.5°C ($F = 66.94$, $df = 2$, $p = 0.024$) (Figure 2). Egg maturation strategy also had a significant effect on amount of lipids at emergence between populations ($F = 4.17$, $df = 4, 76$, $p = 0.002$). Proovigenic populations emerged with more lipids than synovigenic populations/species in all temperature treatments. A significant decrease in amount of lipids occurred with an increase in temperature in proovigenic populations but no difference was detected in synovigenic populations/species.

We did not observe any difference between populations in the slope of reaction norms of amount of sugars between 20°C-22.5°C, 22.5°C-25°C ($F = 7.78$, $df = 2$, $p = 0.49$) but sampling site explained differences between 25°C-27.5°C ($F = 34.18$, $df = 2$, $p = 0.043$). Females from the desert area had a flatter reaction norm than other populations between these two temperatures (Figure 2b). The sampling site had and effect on amount of sugars only at

27.5°C (p = 0.021). Females from the mild climate emerged with more sugars than desert females at this temperature but no difference occurred between the three other temperatures.

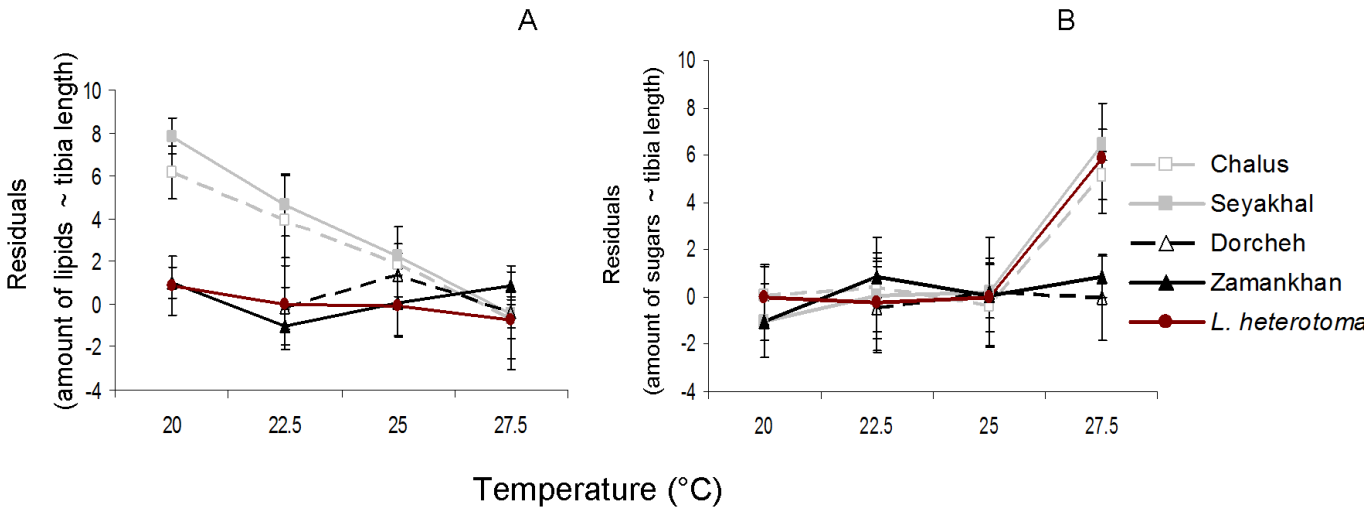

Figure 2. Residuals of models comparing (A) amount of lipids and (B) amount of sugars at emergence with tibia length as covariate, in response to four developmental temperatures. One model was tested per population. The higher the residuals, the more females had nutrients for a same body size. Errror bars: ± SE.

Longevity

Longevity significantly decreased with an increase in temperature in all populations but the slope of reaction norms was affected by the interaction sampling site* egg maturation strategy between 20°C-22.5°C, 22.5°C-25°C (F = 72.99, df = 2, p = 0.014), 25°C-27.5°C (F = 63.14, df = 2, p = 0.022). Reaction norms were flatter in proovigenic populations and intermediate in *L. boulardi* synovigenic populations (Figure 3).

The interaction climate* egg maturation strategy had a significant effect on longevity for the three coldest temperatures (20°C: χ^2 =95.18, p < 0.001; 22.5°C: χ^2 = 47.04, p = 0.02; 25°C: χ^2 =35.67, p = 0.038). *L. heterotoma* females lived longer than *L. boulardi* wasps, and desert females of this species lived longer than mild females. At 27.5°C, only climate of origin had

an effect on longevity (χ^2 =41.68, p = 0.029), females from the mild climate living shorter than desert populations at this temperature.

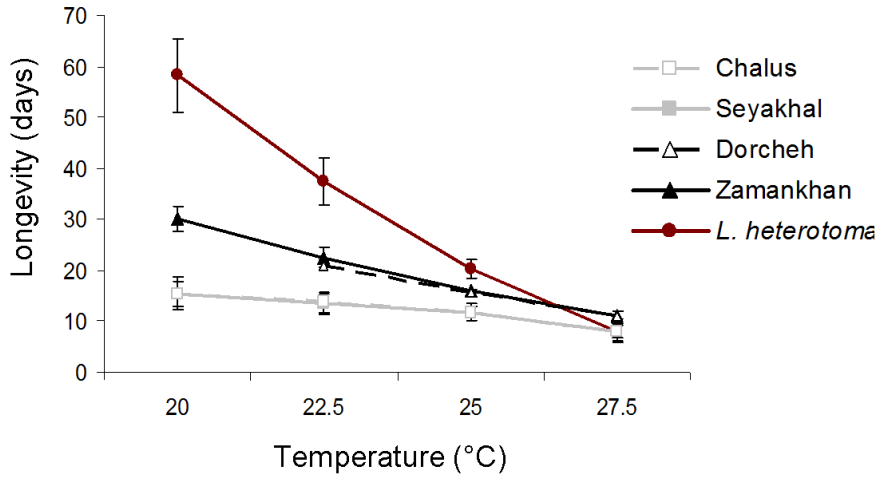

Figure 3. Longevity of fed females measured at four developmental temperatures. Error bars: ± SE.

Discussion

We observed different reaction norms between populations for several traits, suggesting that different levels of phenotypic plasticity have been selected. Our results provide evidence that reaction norms of reproduction-related traits have been mainly selected in response to environment of origin and not egg maturation strategy, as we found similar curves for egg load and egg size in *L. boulardi* and *L. heterotoma* from a same climate. The reaction norms of amount of sugars also suggest that different levels of phenotypic plasticity have been selected in different environments. However, results on longevity and amount of lipids suggest that life histories may also play a role in the selection of reaction norms.

Environment as explanatory variable. Egg load and egg size were similar from 20°C to 27.5°C in synovigenic populations of *L. boulardi* from the desert area whereas they were plastic in proovigenic populations of *L. boulardi* and synovigenic population of *L. heterotoma*

from the mild climate. Contrarily to our hypothesis on the role of egg maturation strategy on evolution of plasticity, initial egg load was not buffered against thermal variations in proovigenic populations. Environment of origin is more likely to explain differences in the level of plasticity for these traits. The desert area is characterised by more variable climate and host distribution than the mild area (Moiroux *et al.* 2010). As suggested by Wagner *et al.* (1997), canalisation in fitness-related traits may occur there, to buffer fitness in variable environment. Egg load and egg size would be maximised even in stressful conditions commonly occurring in the desert region. This result is in agreement with the observations of Moiroux *et al.* (submitted), who observed in *L. boulardi* a lower level of phenotypic plasticity in egg load when comparing populations from forests (i.e. a highly variable host distribution) and orchards (i.e. a less variable host distribution). Liefting *et al.* (2009) also observed a similar result for developmental rate in *Drosophila serrata* when comparing populations from variable and more stable climates. Environmental variation may thus be more important than life histories in the selection of reaction norms.

We observed opposite patterns in thermal plasticity for egg load and egg size in mild climate. Egg load decreased with the increase in temperature while their volume increased from 20°C to 25°C and decreased at 27.5°C. Our results agree with the negative correlation found between these two reproductive traits in butterflies or flies, i.e. primary consumers, reared at different temperatures (Blanckenhorn 2000, Ernsting & Isaaks 1997, Ernsting & Isaaks 2000, Fischer & Fiedler 2001, Fischer *et al.* 2003). However the thermal plasticity observed in our study is opposite to those described in other studies where an increase in egg number and a decrease in egg volume with an increase in temperature have been observed. The above mentioned authors provided evidence that in tropical species, bigger eggs laid at low temperatures, a non-optimal condition, correlated with a higher hatching success. We may thus also expect that plasticity in egg size has been selected in mild populations to increase hatching success in stressful conditions. However our species also originated from hot climates and are frequently exposed to temperatures close to 25°C. We can thus hardly conclude that this plasticity in egg size has been selected to increase hatching success in stressful condition. When temperature increases, drosophila larvae are smaller. They thus provide fewer resources to the parasitoid. Laying bigger eggs with more resources, traded-off against fewer eggs, may thus be adaptive at this high temperature as it would compensate for the fewer nutrients provided by the host. This hypothesis involves that reaction norms of reproduction-related traits have been selected in response to the body size reaction norm of host and not in response to the environment, in the populations from the mild climate. The

decrease in egg load and egg volume at 27.5°C suggests that this temperature is stressful for populations from mild climates, and development in hot conditions may be paid by a lower allocation of resources to reproduction and maintenance.

Differences in amount of sugars also supports the hypothesis that environmental variation plays a major role in the selection of reaction norms. We did not observe any difference in amount of sugars, except an increase at the hottest temperature in mild populations of *L. boulardi* and *L. heterotoma*. Carbohydrates, and particularly sorbitol and trehalose, are involved in desiccation and heat resistance, as they protect proteins from thermal denaturation (Henle & Warters 1982, Wimmer *et al.* 1997). A greater amount of sugars may thus be adaptive at higher temperature, as found in whiteflies by Salvucci (2000). Parasitoids may thus adjust the balance of nutrients before emergence, while feeding on the larva. However, this result may also be explained by the better hatching success of individuals with a high amount of sugars at high temperature in populations from mild climates, as suggested by the lower emergence rate observed at this temperature (unpublished data).

Egg maturation strategy as explanatory variable

Egg maturation strategy affected thermal plasticity in amount of lipids and longevity but sampling site did not. It is likely that differences in lipogenesis ability more than egg maturation strategy explain differences for these traits. We expected a flatter reaction norm in proovigenic populations unable to synthesise lipids, which will be limited during adult life to the nutrients accumulated during larval life. However the quantity of lipids decreased with increasing temperature in these populations whereas the amount of lipids was similar for synovigenic populations of *L. boulardi* and *L. heterotoma* between the four populations. Even if amount of lipids decreased with increasing temperature in proovigenic populations, it was always higher at emergence than the amount of synovigenic populations able to synthesize lipids during adult life. An explanation may be that there is no thermal plasticity in amount of lipids in proovigenic populations and that this trait is maximised according to the nutrients provided by the host, as a lower lipid content occurs at higher temperature in *Drosophila* (Schmidt *et al.* 2005).

Longevity reaction norms were flatter in *L. boulardi* proovigenic populations than in any other population. We observed an increase in longevity with a decrease in temperature in all populations, as found in the great majority of studies (Nylin & Gotthard 1998), but that increase was stronger in synovigenic populations/species. These differences are probably the

consequence of differences in lipogenesis ability. At low temperature, nutrients are allocated slowly (Gillooly *et al.* 2001) and may affect the longevity, whereas they are consumed faster at higher temperature. Lipogenesis ability observed in *L. heterotoma* and synovigenic populations of *L. boulardi* may thus allow a strong increase in longevity at low temperature whereas its absence does not allow it in proovigenic populations. These differences in lipogenesis may also explain the shorter lifespan in these proovigenic populations whereas the longest lifespan of *L. heterotoma* may be explained by the lower metabolic rate of this species (unpublished data).

In this study, the adaptive importance of phenotypic plasticity in longevity and amount of lipids are unclear as thermal variations in these traits are likely to be the only result of physiological constraints (van Voorhies 1996).

Conclusion

Intra- and interspecific variations in level of phenotypic plasticity have been rarely investigated, especially when considering life history as explanative factor. In this study, we observed differences in level of plasticity between species and populations, but the reproductive strategies of species/populations did not explain these differences, whereas the sampling site did. Our study suggests that environmental factors, such as climate or resource distribution, may play a more important than intrinsic characteristics in the selection of the slope of reaction norms in parasitic wasps.

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Chapitre 4.

Discussion générale

Discussion générale

1- Le climat, un facteur essentiel dans l'évolution des histoires de vie des parasitoïdes ?

Un des nouveaux défis de l'écologie évolutive est de prédire l'évolution des histoires de vie des organismes en réponse au réchauffement global actuel et à venir. Pour ce faire, il est essentiel de déterminer comment ces histoires de vie ont évolué et évoluent en réponse au climat. Une des approches principales pour comprendre ces mécanismes consiste à comparer en conditions similaires des populations d'une même espèce prélevées dans différents climats. Chez les ectothermes, les adaptations locales de traits d'histoire de vie, comme le timing de reproduction ou les capacités de résistance aux stress thermiques ont été presque exclusivement associées à des différences de températures moyennes du milieu d'origine (ex : Hoffmann *et al.* 2002, Mitrovski & Hoffmann 2001, Schmidt *et al.* 2005). Ainsi, une durée de vie courte, une teneur en lipides réduite, une petite taille ainsi qu'une reproduction précoce ont généralement été sélectionnées dans les climats les plus chauds (Mitrovski & Hoffmann 2001, Schmidt *et al.* 2005, Schmidt & Paaby 2008). Peu d'attention a été accordée au rôle des divers facteurs biotiques variant localement avec la température, comme la composition des communautés, la structure de l'habitat ou la distribution des ressources dans l'évolution des histoires de vie (ex : Bradshaw *et al.* 2004, Malo & Baonza 2002).

Bien que cette thèse s'intéresse à un niveau trophique supérieur à celui considéré dans la majorité des études clinales, à savoir des consommateurs primaires, nous pensions observer chez le parasitoïde *Leptopilina boulardi* des variations géographiques proches de celles décrites ci-dessus, puisque la température devrait affecter directement et invariablement le métabolisme et la physiologie des insectes (Gillooly *et al.* 2001).

Si des variations géographiques des traits d'histoire de vie existent bien en Iran (article 1), celles-ci se sont avérées opposées à ce qui avait été décrit dans d'autres études, c'est-à-dire une reproduction précoce et une vie courte en milieu chaud. Les différences de photopériode existant entre les sites échantillonnés, mais non intégrés dans nos études, pourraient potentiellement expliquer les différences observées. Néanmoins, ce sont les variations géographiques de distribution des hôtes qui semblent les plus à même d'expliquer les adaptations locales observées, comme de la synovogénie et une capacité de lipogenèse dans

les populations du milieu chaud. Toutefois, l'absence de données sur cette variable environnementale ne permet pas de tester cette hypothèse et de discriminer ce facteur biotique des facteurs climatiques.

Alors qu'en Iran la distribution des hôtes pourrait expliquer les variations géographiques d'histoires de vie, la compétition interspécifique expliquerait celles observées dans la vallée du Rhône (article 3). Sur une distance de 50 kilomètres, pour laquelle le climat reste similaire, nous avons observé chez deux espèces de parasitoïdes, *Asobara tabida* et *Leptopilina heterotoma*, des variations d'histoires de vie associées à une présence récente ou non de l'espèce compétitrice invasive *L. boulardi*. Cette évolution en réponse aux facteurs biotiques et non en réponse au climat, contraire à ce qui est observé chez les consommateurs primaires, est probablement le fait du lien direct qui existe entre le nombre d'hôtes rencontrés et le nombre de descendants chez les parasitoïdes (Wajnberg *et al*. 2008). La disponibilité des hôtes, dépendante de leur distribution et de la compétition interspécifique, jouerait alors un rôle majeur dans l'évolution des histoires de vie de ce groupe, plus important que le climat lui-même. Ces adaptations locales pourraient donc être sélectionnées à une échelle bien plus fine que celles généralement considérées dans ce type d'étude, où des populations sont échantillonnées à l'échelle d'un continent (Ellers & van Alphen1997, Hoffmann *et al.* 2002, Schmidt & Paaby 2008). Les mesures, effectuées en conditions standard en laboratoire, avec notamment un apport *ad libitum* de nourriture ou une absence de vols pour des insectes se déplaçant en grande partie grâce à ce moyen de locomotion, obligent cependant à la prudence. Des résultats bien différents pourraient ainsi être observés *in natura*, avec par exemple des valeurs de traits plus faibles en désert où la nourriture pourrait être moins abondante.

Tous nos résultats suggèrent que l'évolution des espèces du troisième niveau trophique serait bien plus complexe que de simples modifications physiologiques en réponse à une hausse des températures, comme une réduction de la longévité et une reproduction précoce. Les modèles de prédictions d'évolution des histoires de vie en réponse au réchauffement climatique devraient intégrer autant que possible l'incidence des changements climatiques annoncés sur les facteurs biotiques, et non un impact direct du climat. L'augmentation des épisodes de sécheresse annoncée dans les régions subtropicales pourrait par exemple limiter la quantité de fruits disponibles pour les drosophiles, et donc la disponibilité en hôtes pour les parasitoïdes de drosophiles. De plus, les modifications de traits d'histoire de vie pourraient être bien plus importantes qu'un simple ajustement de l'allocation des ressources entre les différents traits. Durant cette thèse, des variations intraspécifiques jamais décrites chez les parasitoïdes ont pu être mises en évidence. Nous avons par exemple observé l'existence au

sein d'une même espèce de populations proovogéniques et synovogéniques, ainsi que d'une capacité ou non à synthétiser des lipides durant la vie adulte. Les facteurs biotiques semblent également être responsables de variations géographiques dans les normes de réaction thermiques et le degré de plasticité phénotypique des traits liés à la reproduction et de certains traits physiologiques. Les adaptations locales des traits d'histoire de vie et de leurs normes de réaction observées durant cette thèse, ainsi que les facteurs environnementaux ayant sélectionnés ces adaptations, sont synthétisées en Figure 1.

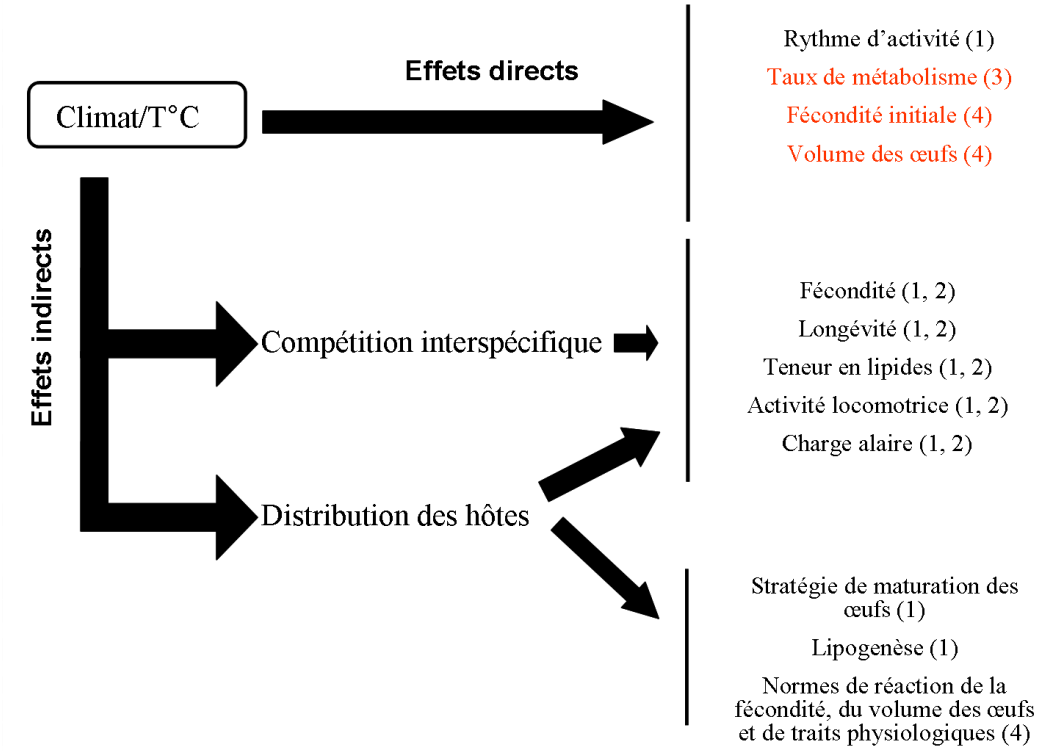

Figure 1. Adaptations locales (noir) et plasticité (rouge) des traits d'histoire de vie décrites dans cette thèse, en réponse aux facteurs abiotiques du climat et de facteurs biotiques dépendants du climat. Les numéros entre parenthèses indiquent les articles correspondant.

Evolution des stratégies de reproduction

Une reproduction précoce, associée à une longévité réduite, est généralement sélectionnée dans les climats chauds (Mitrovski & Hoffmann 2001, Schmidt *et al.* 2005). Contrairement à ces résultats, nous avons découvert chez *Leptopilina boulardi* une longévité supérieure et une reproduction différée (synovogénie) dans nos populations prélevées en Iran en zone désertique, chez une espèce considérée comme proovogénique (Kopelman & Chabora 1986). Ces différences d'histoires de vie ont été associées aux différences géographiques de distribution des hôtes. La zone désertique représente un environnement hétérogène où les parasitoïdes doivent parcourir de longues distances pour trouver des hôtes. Dans cet habitat, il y aurait eu une sélection forte pour des caractères plastiques, comme la synovogénie. Cette stratégie de maturation des œufs permettrait de répondre à la variabilité environnementale, les ressources étant allouées soit à la reproduction, soit à la maintenance ou à la dispersion en fonction des opportunités de ponte (Jervis *et al.* 2001). La synovogénie présente également l'avantage physique de réduire la charge alaire en début de vie, le coût de déplacement étant alors moins important que pour des femelles émergeant avec la totalité de leurs œufs matures. Cette réduction de charge alaire faciliterait les déplacements entre patchs distants (Gilchrist & Huey 2004, Starmer & Wolf 1989). A l'inverse, les femelles des populations de climat plus tempéré émergent avec la totalité de leurs œufs matures. Les modèles proposés par Ellers & Jervis (2003, 2004) suggèrent qu'un milieu uniforme serait le facteur essentiel pour qu'une stricte proovogénie soit sélectionnée, comme observé dans ce climat où les vergers sont communs et représentent un habitat riche. A notre connaissance, cette étude est la première à montrer l'existence de populations proovogéniques et synovogéniques au sein d'une même espèce de parasitoïdes. Nos résultats soutiennent l'idée qu'il existe bien un continuum entre les deux stratégies de maturation (Jervis *et al.* 2001), et qu'une évolution de la proovogénie (observée dans la majorité des populations de *L. boulardi*) vers la synovogénie en milieu hétérogène reste possible et devrait être considérée comme une alternative dans les modèles consacrés à l'évolution des stratégies de reproduction des parasitoïdes. Ces différentes stratégies de reproduction auraient été sélectionnées en réponse à la distance entre patches d'hôtes, dépendante du climat, et non en réponse directe au climat.

Outre la distribution des patches d'hôtes, la compétition interspécifique semble également jouer un rôle essentiel dans l'évolution des histoires de vie des parasitoïdes de drosophiles. C'est ce que nous avons pu observer dans la vallée du Rhône en comparant des populations d'*Asobara tabida* et de *Leptopilina heterotoma* soumises depuis deux (nord de

Lyon) ou douze ans (sud de Lyon) à l'espèce invasive *L. boulardi* (article 3). Pour les deux espèces autochtones, la fécondité des populations du sud s'avère supérieure à celle des populations du nord de Lyon, ce résultat étant confirmé par Fleury *et al.* (2009). Ces observations sont conformes à l'hypothèse de Price (1974) qui prédit une augmentation de la fécondité dans les environnements où la mortalité juvénile est élevée, induite dans notre cas par l'espèce envahissante. Cette adaptation locale permettrait le maintien des populations de *L. heterotoma* et *A. tabida* en présence d'un compétiteur supérieur, bien que l'abondance relative des espèces autochtones reste relativement faible en comparaison de *L. boulardi* durant les périodes où celui-ci est présent (Fleury *et al.* 2009).

Evolution des capacités de lipogenèse et de la teneur en lipides

Les lipides constituent une ressource primordiale chez les parasitoïdes, à la base de nombreux compromis évolutifs entre fécondité précoce, fécondité tardive, longévité ou dispersion (Ellers 1996, Ellers & van Alphen 1997). Les résultats observés dans certaines populations rhodaniennes soulignent ainsi un compromis entre la fécondité initiale et la teneur en lipides dans le corps gras à l'émergence, positivement corrélée à la longévité. Par exemple, la population *Asobara tabida* du sud de Lyon produit plus d'œufs mais vit moins longtemps et possède moins de lipides que celle du nord. Ces nutriments sont d'autant plus centraux dans l'évolution des histoires de vie des parasitoïdes que la majorité des espèces ne peuvent en synthétiser via l'alimentation durant leur vie adulte (Visser & Ellers. 2008). Visser *et al.* (2010) ont été les premiers à décrire des cas de lipogenèse dans ce groupe. Ils ont comparé 24 espèces de parasitoïdes et ont trouvé de la lipogenèse chez 7 d'entre elles, réparties dans trois clades distincts. Ces auteurs proposent que la capacité à synthétiser des lipides ait été regagnée au cours de l'évolution chez certaines espèces généralistes capables de parasiter un grand nombre d'espèces hôtes, comme *Leptopilina heterotoma* pour qui nous avons confirmé cette capacité de lipogenèse dans deux populations iraniennes (article 5, données non présentées). D'après Visser *et al.* (2010), le fait d'être généraliste réduirait l'efficacité de la manipulation et de l'utilisation des lipides de l'hôte et aurait conduit à l'apparition de lipogenèse. Néanmoins, cette hypothèse ne peut expliquer nos différences entre populations iraniennes de *L. boulardi* puisque cette espèce ne parasite que deux espèces de drosophiles du même sous-groupe, *D. melanogaster* et *D. simulans*, toutes deux présentes dans nos trois aires d'échantillonnage. De plus, la capacité de synthétiser des lipides n'est détectée que dans

certaines populations alors qu'elle devrait être observée dans toutes (article 1). Pour cette espèce, la lipogenèse serait donc réapparue au cours de l'évolution en réponse à certaines variables écologiques, vraisemblablement une distribution hétérogène des hôtes. De même que pour la synovogénie, ce type d'adaptation aurait émergé dans un habitat extrême, ici une région désertique. Cette capacité permettrait de s'affranchir des contraintes physiologiques qui seraient induites par une absence de lipogenèse, dans un environnement où les femelles ont besoin d'une grande quantité de lipides pour se déplacer sur de longues distances (Nation 2008) pour trouver des hôtes. Les populations incapables de synthétiser des lipides devraient quant à elles maximiser la quantité de lipides prélevées dans l'hôte pour assurer leur reproduction et leur maintenance, comme observé dans nos populations iraniennes (article 5).

Métabolisme

La quasi-totalité des études comparatives ont montré une « countergradient variation » du taux de métabolisme, c'est-à-dire que des individus ayant un génotype « taux de métabolisme faible » sont retrouvés majoritairement dans des climats chauds et secs, où la température induit un taux de métabolisme élevé (ex : Addo-Bediako *et al.* 2002, Berrigan & Partridge 1997, Mueller & Diamond 2001). Les auteurs ayant mis en évidence un tel pattern géographique proposent qu'un taux de métabolisme élevé aurait été sélectionné dans les zones froides pour compenser l'effet des basses températures sur le métabolisme (Addo-Bediako *et al.* 2002, Lee & Baust 1982) ; ou qu'un métabolisme faible serait sélectionné en milieu chaud et désertique pour limiter les pertes d'eau (Mueller & Diamond 2001). Contrairement à ces études, nous avons observé une « cogradient variation » pour ce trait physiologique (article 3), c'est-à-dire qu'un génotype « taux métabolique élevé » a été sélectionné dans un environnement chaud et sec (Conover & Schultz 1995). Les ressources lipidiques étant moins limitantes dans ces populations du fait de leur capacité à synthétiser des lipides, elles peuvent être allouées plus rapidement entre les différentes fonctions, via un taux de métabolisme supérieur, sans que ceci n'induise nécessairement de baisse de la longévité ou de la fécondité. Un taux de métabolisme élevé permettrait essentiellement de trouver des hôtes plus rapidement et de maintenir une activité locomotrice soutenue dans un environnement hétérogène où de longs déplacements sont requis. Ce résultat constitue à notre connaissance le premier cas de ce « cogradient variation » pour le taux de métabolisme chez des arthropodes, et le premier cas où des différences de caractéristiques intrinsèques, et non environnementales, auraient conditionné l'évolution du taux de métabolisme.

Corrélations entre traits

Dans nos travaux, les compromis habituellement décrits entre traits comme un trade-off entre longévité et fécondité (Bell & Koufopanou 1986, Ellers *et al.* 2000), ou entre fécondité et activité locomotrice (Zhao & Zera 2006) n'ont pas toujours été observés. Par exemple, les femelles de nos populations iraniennes de milieu désertique vivent plus longtemps, se déplacent plus et plus vite tout en ayant autant d'œufs en fin de vie que les femelles des populations de milieu humide (article 1). Cette apparente absence de compromis s'explique vraisemblablement par la capacité de lipogenèse des premières, qui échappent aux contraintes physiologiques auxquelles sont soumises les secondes, incapables de synthétiser des lipides. Une capacité de lipogenèse sélectionnée uniquement dans certaines populations amène ainsi à totalement reconsidérer les histoires de vie et trade-offs, les contraintes physiologiques n'étant plus les mêmes.

De même, dans nos populations rhodaniennes de *Leptopilina heterotoma*, les populations du sud produisent d'avantage d'œufs que celles du nord, tout en vivant aussi longtemps que celles-ci. Dans ce cas, c'est le taux de métabolisme plus faible dans les populations du sud de *L. heterotoma* qui expliquerait que la fécondité plus importante de celles-ci n'induise pas de diminution de longévité, les ressources énergétiques étant allouées plus lentement que dans le nord. Sur ce même gradient géographique, les populations d'*Asobara tabida* du sud produisent également plus d'œufs que celles du nord de Lyon mais vivent moins longtemps, conformément à l'idée d'un compromis entre fécondité et longévité, alors qu'aucune différence du taux de métabolisme n'est détectée. Les contraintes écologiques sont similaires pour les deux espèces mais les différences de contraintes physiologiques impliquent que l'évolution des histoires de vie puisse être différente pour certains traits.

Ces deux résultats soulignent l'importance d'intégrer autant que possible la physiologie des organismes afin de comprendre l'évolution des histoires de vie, tant certains caractères peuvent modifier les contraintes connues.

En milieu naturel, les femelles parasitoides doivent parfois parcourir de longues distances afin de trouver des hôtes viables pour pondre leur descendance. Chez les insectes, le vol est un moyen de déplacement extrêmement coûteux, qui pourrait avoir d'importantes conséquences dans l'allocation des ressources dans les traits d'histoire de vie, et notamment la reproduction ou la longévité. Durant nos travaux, les femelles parasitoïdes ne pouvaient effectuer de réels vols dans les pots d'élevage utilisés, biaisant très certainement les résultats observables dans la nature. Il est en effet vraisemblable qu'*in natura*, le coût des

déplacements puisse profondément modifier les valeurs de traits observés durant cette thèse, avec certainement une longévité ou une fécondité plus faibles dans les populations issues des milieux où les femelles doivent voler sur de longues distances, comme les régions désertiques.

2- Plasticité phénotypique : adaptations, variations géographiques

La plasticité phénotypique est la capacité d'un organisme à produire différents génotypes dans différents environnements (Bradshaw 1965). Elle peut être représentée par une norme de réaction (de Jong 1990), c'est-à-dire une fonction où la variable environnementale est représentée en abscisse et la valeur du trait étudié en ordonnée. Cette norme de réaction est caractérisée par trois éléments, qui présentent tous une variation génétique et donc un potentiel adaptatif (Gutteling *et al.* 2007, Liefting & Ellers 2008, Liefting *et al.* 2009, Scheiner 1993) : l'élévation, la gamme environnementale et la pente. Dans la seconde partie de cette thèse, nous avons cherché à déterminer (1) si les traits d'histoire de vie sont plastiques et si cette plasticité thermique présente un avantage évolutif, et (2) quels facteurs, environnementaux et intrinsèques à l'organisme, peuvent conditionner les normes de réaction. Nous avons notamment intégré la variabilité environnementale et les pressions de sélection liées à l'opposition proovogénie/synovogénie pour comprendre comment la force de la plasticité, représentée par la pente de la norme de réaction, a évolué.

Plasticité adaptative ?

D'après nos mesures de normes de réaction, de nombreux traits d'histoire de vie semblent varier avec la température. Le premier facteur, central dans la théorie des histoires de vie, est le taux de métabolisme. Celui-ci augmente avec la température de développement et d'exposition pour nos quatre populations iraniennes (article 4). Cette variation est similaire à ce qui a été décrit dans plusieurs études empiriques et modèles intégrant les principes de thermodynamique et de cinétique (Gillooly *et al.* 2001, Chown 1997, Nespolo *et al.* 2007). Néanmoins, et contrairement aux prédictions de ces modèles, on observe une diminution de ce taux entre 25°C et 27,5°C, lorsque la température devient trop stressante. Il semblerait donc que, contrairement à l'augmentation linéaire suggérée dans les travaux de modélisation entre des températures acceptables pour les organismes classiques, soit entre 0°C et 40°C, le

taux de métabolisme puisse décroître bien avant les 40°C. Cette diminution, associée à une mortalité rapide à 27,5°C, suggère que des dégâts ou une inactivation des fonctions physiologiques apparaissent rapidement dès que la température moyenne est trop élevée.

Cette augmentation du taux de métabolisme avec la température expliquerait en partie la diminution de longévité et de la teneur en lipides observées aux températures élevées dans nos populations françaises, espagnoles et iraniennes (articles 2 et 5), et décrites dans de nombreuses autres études (Colinet *et al.* 2007, Nylin & Gotthard 1998). Les variations de ces traits physiologiques avec la température peuvent difficilement être considérées comme un ajustement adaptatif (Pigliucci 2005) des traits en réponse à la température puisqu'elles sont certainement le seul fait de contraintes physiologiques associées à la température. Les variations de quantité de lipides observées dans nos populations proovogéniques d'Iran laissent néanmoins supposer que la quantité de lipides pourrait être maximisée et serait conditionnée par ceux fournis par l'hôte, dont la taille diminue avec une augmentation de la température.

Les variations du nombre et de la taille des œufs semblent quant à elles adaptatives, et correspondraient à un ajustement optimal des traits en réponse à la température. Dans nos populations iraniennes de milieu humide, un compromis entre nombre et volume des œufs semble exister pour ces populations proovogéniques entre 20°C et 25°C. Nous avons en effet constaté une diminution du nombre d'œufs et une augmentation de leur volume avec une augmentation de la température. Ces variation du nombre et du volume des œufs avec la température vont à l'opposé de ce qui a été décrit jusqu'à présent chez certaines mouches et papillons (Blanckenhorn 2000, Ernsting & Isaaks 2000, Fischer *et al.* 2003, Stillwell & Fox 2005), pour lesquels le volume des œufs augmente avec une diminution de la température alors que leur nombre diminue. Les auteurs de ces études ont montré que de telles normes de réaction présentent un avantage évolutif. Des œufs de grande taille, pourvus de plus de ressources énergétiques, maximiseraient le succès d'éclosion à basse température, non-optimale pour les espèces tropicales étudiées. Nos populations iraniennes de *Leptopilina boulardi* pour lesquelles les normes de réaction ont été mesurées sont fréquemment confrontées à des températures élevées. Il est donc peu probable que des œufs plus gros soient produits à 25°C afin d'augmenter le succès d'éclosion à une température stressante. Chez les drosophiles comme chez la majorité des insectes, il existe une corrélation négative entre température et taille des individus. Des larves se développant à une température élevée procureraient donc une quantité moindre de ressources au parasitoïde. La production d'œufs plus gros, avec plus de ressources, à une température élevée permettrait de contrebalancer la

plus faible quantité de nutriments prélevés dans l'hôte et de maximiser la fitness des descendants. La norme de réaction de la taille des œufs aurait donc été sélectionnée en réponse à la norme de réaction de la taille des larves de drosophiles et non en réponse à la température.

Force de la plasticité

Parmi les trois éléments de la plasticité phénotypique qui présentent un potentiel évolutif, la force de la plasticité est celle qui présente le plus de variations entre nos populations.

La fécondité représente la composante principale de la fitness des parasitoïdes. Nous avons observé une canalisation (Stearns *et al.* 1995) de la fécondité initiale dans les populations de milieu désertique en Iran. Le nombre d'œufs est en effet quasiment identique entre les différentes températures alors qu'il varie fortement pour les populations de milieu humide en Iran. Contrairement à nos prédictions, nous n'avons pas détecté de variations de la force de la plasticité associées à la stratégie de maturation des œufs (proovogéniques ou synovogéniques). Les facteurs environnementaux semblent donc jouer un rôle primordial dans la sélection des pentes de normes de réaction (Aubret *et al.* 2004, Liefting *et al.* 2009, Pfennig & Murphy 2002). Ces résultats sont en accord avec les prédictions d'une canalisation des traits fortement liés à la fitness en milieu variable (Richards *et al.* 2006, Stearns & Kawecki 1994, Wagner *et al.* 1997), si l'on considère la distribution des hôtes ou le climat comme facteurs explicatifs. Cette canalisation permettrait de tamponner la fécondité face aux fortes variations environnementales, et de limiter les fortes diminutions de fitness en cas de conditions éloignées de l'optimum. Cette canalisation serait à la fois environnementale et génétique puisque plusieurs génotypes testés ont été utilisés pour établir nos normes de réaction. Nos résultats sont en accord avec Liefting & Ellers (2008) qui ont observé une canalisation du taux de croissance dans des populations de collemboles prélevées en bruyère (climat variable) en comparaison de populations issues de forêts, au climat plus stable. L'augmentation de la variabilité climatique annoncée (GIEC 2007) pourrait donc avoir un impact important sur les capacités plastiques des ectothermes.

Travaux en cours et Perspectives

Durant cette thèse, nous avons observé que le rôle des facteurs biotiques dans l'évolution des histoires de vie des parasitoïdes est vraisemblablement plus important que celui du climat lui-même. Néanmoins, l'absence de données précises de terrain quant aux facteurs biotiques tels que la distribution et l'abondance des hôtes et des ressources alimentaires, ou certains facteurs abiotiques, comme la photopériode ou même le microclimat, ne permettent pas de réellement tester l'influence de chacun des ces facteurs. Une première étude dans laquelle nous avons comparé des populations prélevées en vergers ou forêts sous différents climats nous a permis de confimer l'importance de l'habitat, et notamment de la distribution des hôtes, en comparaison du climat régional. Une étude comparative d'un grand nombre de populations intégrant autant que possible les différents facteurs abiotiques (stations météorologiques sur chaque site) et biotiques (relevés de terrain) d'origine s'avère cependant nécessaire afin de discriminer le rôle de chacun de ces facteurs dans l'évolution des histoires de vie des parasitoïdes.

Chez les insectes, le vol est un moyen de déplacement extrêmement coûteux, qui pourra avoir d'importantes conséquences dans l'allocation des ressources entre différents traits. Durant cette thèse, les femelles parasitoïdes ne pouvaient effectuer de réels vols dans nos pots d'élevage trop exigus, alors que ces vols, nécessaires pour trouver des patchs d'hôtes, pourraient modifier les différences observées entre populations. Nous testons actuellement les conséquences de trajets inter-patchs, en faisant varier les distances parcourues grâce à des moulins de vol, sur d'autres traits tels que l'indice d'ovogénie, la teneur en lipides et la longévité. De même, nous testons le postulat selon lequel une charge alaire plus faible facilite le vol entre patches et aurait pu ainsi être sélectionné en réponse à la distribution des hôtes.

Au cours de ce travail, nous avons également noté la non-détection de certains trade-offs connus, comme celui entre fécondité et longévité (Calow 1979) en comparant certaines populations. Nous avons associé cette absence de détection de compromis dans ces populations à leur capacité de lipogenèse, dont la variation intraspécifique a été décrite pour la première fois chez les parasitoides dans cette thèse, ou à un faible taux de métabolisme (article 3), qui réduirait la vitesse d'allocation des ressources. Ces traits physiologiques, rarement considérés, pourraient donc modifier les prédictions d'évolution des histoires de vie. Néanmoins, ces seules corrélations phénotypiques à l'échelle inter-populationnelles ne permettent pas de conclure quant à l'absence réelle de trade-offs (Roff 1992). Afin de prouver

l'importance de ces deux caractères physiologiques dans l'évolution des histoires de vie et des trade-offs, il nous faut manipuler expérimentalement ou sélectionner un trait comme la fécondité dans des populations capables ou non de lipogenèse, ou ayant un taux de métabolisme plus ou moins élevé, afin de mesurer leur impact sur les traits généralement associés à la fécondité comme la longévité ou la reproduction future.

Les variations inter-populations extrêment fortes observées dans certaines populations, comme l'existence de lipogenèse ou la transition proovogénie/synovogénie chez *Leptopilina boulardi*, suggèrent des pressions de sélection suffisament fortes pour faire émerger ce type d'adaptations au sein des populations. Des travaux de modélisation, intégrant des approches issues de la théorie des histoires de vie, de l'écologie de la dispersion, de la physiologie et de l'optimal foraging sont actuellement en cours de développement afin de comprendre quels facteurs (température, amplitude thermique, distribution des hôtes, richesse des patchs) et quelle intensité de ces facteurs ont pu conduire à la sélection de synovogénie chez une espèce majoritairement proovogénique.

Enfin, nous avons constaté que le niveau de plasticité phénotypique de certains traits aurait été sélectionnée en réponse à certains facteurs biotiques, comme la taille des hôtes, dépendante de la température. Nous avons ainsi observé une diminution du nombre d'œufs des parasitoïdes et une augmentation de leur taille avec une augmentation de la température, à l'opposé des nombreux travaux ayant testé la plasticité thermique de ces traits chez les insectes (Blanckenhorn 2000, Fischer *et al.* 2003). Des œufs plus gros seraient pondus à température élevée pour compenser la moindre quantité de ressources de l'hôte. Afin de confirmer l'aspect adaptatif de la plasticité phénotypique, nous avons testé la réversibilité de celle-ci pour ces traits (Ghalambor *et al.* 2007). Pour ce faire, des individus ont été transférés à l'émergence et à cinq jours à des températures différentes de celles de développement. Un réajustement du volume des oeufs avec la température est attendu dans toutes les populations, alors qu'un changement du nombre d'oeufs devrait être observé uniquement dans les populations synovogéniques. Le réajustement du volume des œufs chez les proovogéniques devrait se traduire par une quantité plus importante de ressources stockées dans le corps gras de la mère.

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Annexe

Influence of temperature and parasitism by *Asobara tabida* on larval pupation behaviour in two *Drosophila* species

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Annexe

Abstract

In insects, pupation site selection behavior has large consequences for survival. Here we investigate the combined effect of temperature and parasitism by the parasitoid *Asobara tabida* on larval pupation behavior in two of its main *Drosophila sp.* hosts differing in their climate origin. We found that larvae of *Drosophila melanogaster* - from (sub) tropical regions - placed at 25°C pupate higher in rearing jars, *i.e* where conditions are drier, than those placed at 15°C. The opposite pattern was observed for *D. subobscura* larvae - from temperate regions - which pupate lower, *i.e* on or near the substrate in relatively moist conditions, than those placed at 15°C. When placed at 25°C, parasitized larvae of the two species pupated more closely to the substrate than unparasitized ones. Moreover, *Drosophila* larvae that had been exposed to *A. tabida* but were not parasitized pupated lower than control unparasitized larvae. Both stung larvae and parasitized larvae differed from control ones in their pupation behavior. These results provide new insights into previous findings of host behavior manipulation by *A. tabida* larvae.

INTRODUCTION

The abiotic and biotic conditions experienced by organisms influence their geographical and habitat distribution directly (e.g. Gaston 2003, 2009; Schnebel and Grossfield 1992). Temperature and parasitism are examples of such environmental conditions and both have major effects on life-history traits of organisms. Temperature affects life history via physiology, ecology and so influences fitness of ectotherms (Dillon et al. 2009). Extreme temperatures are injurious and potentially lethal, but even temperature within those lethal limits impacts performance and ultimately individual fitness (Dillon et al. 2009). Parasitoids are common natural enemies of many insect species. As their successful attacks kill the host, the latter is expected to be under strong selection to evolve means of defending itself (Kraaijeveld and Godfray 2003). Parasitoids display a large variety of mechanisms permitting them to manipulate their hosts' behavior and/or physiology to the benefit of their own development (for review, see Beckage and Gelman 2004).

Since insect pupae are immobile, they remain exposed to potentially harmful factors (desiccation, predation, hyperparasitism by pupal parasitoids, fungal infection, etc.) for varied periods of time. Pupation site selection during the late larval stage can thus be critical for survival (Schnebel and Grossfield 1992), and in this way, this trait is supposed to be under strong selection.

The pupation behavior of larvae has been well documented in *Drosophila* species. A number of environmental factors, studied independently for the greater part, including temperature (Schnebel and Grossfield 1992; Vandal and Shivanna 2007), light or darkness (Markow 1979), moisture (Sameoto and Miller 1968), density (Sameoto and Miller 1968; Joshi and Mueller 1993), the presence of predators or parasitism (Seyahooei et al. 2009) have been shown to influence the pupation behavior of *Drosophila* larvae. When tested independently, the effects of each parameter on larval pupation-height responses differ for each species studied. Schnebel and Grossfield (1986, 1992) have underlied the complexity of the relation between temperature and pupation height. The response of a species in pupation-height at one temperature extreme cannot be used to predict responses at the other temperature extreme, and temperature effects on pupation height in one species do not consistently reflect the effects on other species within the *Drosophila* group.

A recent study of Seyahooei et al. (2009) found that attack of larvae of the same host (*Drosophila melanogaster*) by five species of a parasitoid (*Asobara sp.*) affects host pupation height in different ways. The authors hypothesized that *Asobara sp.* are able to manipulate the

behavior of *D. melanogaster* larvae to achieve their own optimal pupation strategy, because it is the host larva that choses the pupation site. In fact, parasitoids and hosts may often differ in optimal pupation strategies. This is especially true for parasitoids spending more time in the puparium than their host (as *Asobara sp.*), thus vulnerable to dangers for a longer time. Seyahooei et al. (2009) did not consider the influence of a particular parasitoid species on pupation height of different host species.

Drosophila larvae are under attack of several parasitoid species, of which the most common in Europe are the braconid *Asobara tabida*, and the figitids *Leptopilina heterotoma* and *L. boulardi* (Carton et al. 1986). Within *Asobara tabida*'s range, most of the potential host species belong to the *obscura* and the *melanogaster* group (Kraaijeveld and van der Wel 1994). While *Drosophila* larvae are pupating, *Asobara tabida* is still completing its development inside the host.

The pupation behavior of larvae has been well documented in species belonging to the *melanogaster* group (*e.g. D. melanogaster, D. simulans*, etc.) (Sameoto and Miller 1968; Schnebel and Grossfield 1992; Casares et al. 1997; Hodge and Caslaw 1998; Vandal et al. 2008; Seyahooei et al. 2009; Beltrami et al. 2010), but has been much less studied in species belonging to the *obscura* group (*e.g. Drosophila subobscura*, *D. pseudoobscura*, etc.) (Markow 1979; Gaso et al. 1988). Moreover, to our knowledge, the combined effect of several parameters on pupation height has never been documented.

The aim of the present work was to investigate the effects of temperature and parasitism as well as their interaction, on the pupation behavior of larvae, using a parasitoid, *Asobara tabida*, and its two main hosts, which differ in climate origin: in Northwestern Europe (*i.e* in temperate regions), *Drosophila subobscura* Collin is the most abundant host species, while in mediterranean regions, *D. melanogaster* Meigen is the main host (Mollema 1988)

We expected that i) temperature would affect the pupation site selection of the two *Drosophila* species differently, because of their different climate origin. At a higher temperature, the risks associated with desiccation should result in a decrease of *D. subobscura* pupation height and a selection of pupation sites near the substrate in relatively moist conditions. *D. melanogaster* is expected to be more resistant to desiccation, and its larvae should be less constrained in choosing pupation sites. Hence, we expect them to pupate further away from the substrate than *D. subobscura* larvae at the same temperature, ii) because of the differences in selection due to differences in the time spent within the puparium, the effect of temperature on pupation behavior differs between hosts and parasitoids. At higher temperature, parasitized hosts need to be protected from desiccation for

a longer period of time and are, therefore, expected to pupate closer to the substrate than unparasitized ones. iii) If parasitoid larvae are able to manipulate host pupation behavior to their own advantage (Seyahooei et al. 2009), parasitized host larvae might choose a different pupation site than unparasitized larvae. We expect *A. tabida* to affect pupation site behavior of its two main hosts in the same way. iv) Finally, we compared pupation behavior of unparasitized larvae with unparasitized ones that had been exposed to the parasitoid, to study if such exposure changes pupation site choice.

Material and Methods

Hosts and parasitoid strains

Origin

Drosophila subobscura and *Drosophila melanogaster* (Diptera: Drosophilidae) develop in fermenting substrates. There are three larval instars before the pupation, but development time of *D. subobscura* is longer than that of *D. melanogaster*. Development time also varies with temperature (*e.g.* 2 weeks at 25°C for *D. melanogaster vs.* superior to 4 weeks at 15°C). They also differ in climate origin: *D. subobscura* originates from temperate European regions whereas *D. melanogaster* has an African, subtropical origin. *D. subosbcura* is found throughout Europe, and has colonized the West Coasts of North and South America while *D. melanogaster* has become a cosmopolitan species (Kraaijeveld and Godfray 1999). *Asobara tabida* (Hymenoptera: Braconidae) is an holarctic solitary endoparasitoid that attacks *Drosophila* larvae feeding on fermenting substrates (Kraaijeveld and Godfray 1997). Its main host species are *D. subobscura* and *D. melanogaster*. Hosts and parasitoids were collected in the Netherlands in 1960 (*D. melanogaster;* Leiden), 1980 (*D. subobscura*; Leiden) and in 2007 (*A. tabida;* Woerdense Verlaat: 52°8’60 N; 4°52’0 E).

Rearings

Hosts were reared in glass jars (h= 13cm, Ø= 6cm) on Agar-yeast-Nipagine substrate with a thick yeast layer. Adult parasitoids were maintained in glass jars (h= 8cm, Ø= 5cm) on Agar-Nipagine substrate and fed *ad libitum* with diluted acacia honey. Since their establishments, *D. melanogaster* and *D. subobscura* colonies have been cultured at 25°C and 20°C respectively. In our experiments, both hosts and parasitoids were reared at 20 ± 1°C, RH= 60 ± 10%, 16L:8D.

Pupation height experiments

To test whether *A. tabida* affects the pupation behavior of its two main hosts in the same way, we set up an experiment using the two host species (*D. subobscura* and D. *melanogaster*) tested independently with the parasitoid, and a control group of non-parasitized hosts for each species. Each of the four combinations consisted of 20 replicates. In each experiment 20 second instar *Drosophila* larvae, reared at 20°C, were offered on a thin yeast suspension and placed in a jar (h= 13cm, Ø= 6cm) containing a layer of agar medium. This is the preferred host stage for oviposition by *A. tabida* females (van Alphen and Drijver 1982). The vials were stoppered with foam bungs. One mated parasitoid female was then placed in each jar and allowed to parasitize hosts during two hours before being removed. The control vials were left without parasitoids.

The effect of temperature was studied by placing 10 replicates at 15°C and at 25°C, for each combination (*i.e.* for each species, jars with unparasitized or parasitized larvae).

All the jars were kept at 15°C or 25°C in Sanyo incubators MIR 253 (RH= 60 ± 10%, 16L:8D) until hosts had pupated.

Measurements

Pupae were located both near the medium in the middle of the jar or on the sides of the jar. Pupation height was measured as the distance (mm) between the surface of the medium and the pupae location (Sokolowski and Hansell 1983). The parasitism status of pupae was systematically checked by dissection (in Ringer's solution) after the experiment.

Statistical analyses

As pupation height is bounded below (larvae cannot pupate lower than the medium) as well as above (they cannot pupate higher than the top of the jar), standard linear parametric models were unsuitable for the analysis of this measure. Since data distributions were similar in shape to Weibull, we applied a Weibull parametric survival regression analysis to our data, and we included the random jar effect as frailty in this model, as done by Seyahooei et al. (2009). Analyses were performed using R 2.7.0 (Ihaka and Gentleman 1996).

Results

Pupation height of host larvae differed significantly according to temperature, host species and parasitism status (Survival regression analysis, $\chi^2_{\text{temperature}} = 98.37$; $\chi^2_{\text{host species}} = 67.94$; $\chi^2_{\text{parasitism status}} = 32.75$, DF = 1, $p < 0.001$).

The two host species responded very differently to temperature. Post hoc z-tests revealed that all *D. melanogaster* larvae placed at 25°C pupated significantly higher than those placed at 15°C (z= 20,11, p<0,001), whereas a higher temperature (25°C) resulted in *D. subobscura* larvae to pupate lower than those placed at 15°C (z= -5,76, p<0,001) (Fig. 1).

Parasitized and unparasitized larvae of the two host species showed similar pupation heights when placed at 15°C (*D. melanogaster*: z= -0,91, p=0,36; *D. subosbcura* : z=-0,9, p=0,36). However, when placed at 25°C, larvae parasitized by *A. tabida* pupated significantly lower than unparasitized ones, in both host species (*D. melanogaster*: z= 4,3, p<0,001; *D. subosbcura*: z=9,29, p<0,001).

The strength of the response to a higher temperature depended on whether or not larvae have been parasitized. The increase in pupation height observed in *D. melanogaster* at 25°C is lower for parasitized larvae, and the decrease in pupation height observed in *D. subobscura* larvae placed at 25°C is stronger in parasitized larvae.

Pupation height was also affected in the larvae that were exposed to the wasp, but had not been parasitized, as they pupated significantly lower than larvae from the control group (excepted for *D. melanogaster* larvae placed at 15°C) (Fig. 1).

Discussion

Choosing a favorable location for pupation may be important for *Drosophila* pre-adult survival as pupae are immobile (Sameoto and Miller 1968; Dillon et al. 2009). Pupation heights of host larvae placed at 15°C differed significantly from those placed at 25°C. Our results are consistent with previous studies that showed that temperature determines pupation height in *Drosophila* larvae (Rodriguez and Sokolowski, 1987; Schnebel and Grossfield, 1986; 1992). In our experiments, the effect of temperature on larval pupation behavior differed between species. *D. melanogaster* and *D. subobscura* larvae placed at 25°C pupated respectively higher and lower than those placed at 15°C. Previous studies have reported that closely related species differed in their larval pupation behavior (Sameoto and Miller 1968; Casares et al. 1997; Godoy-Herrera and Silva-Cuadra 1998; Vandal et al. 2008). Moreover, Schnebel and Grossfield (1992) have documented that the effects of temperature on pupation

height response differed within and among species, and that correlating pupation height observed in laboratory with ecological characters or phylogenetic history is not always easy. The two host species tested differed in climate origin: *D. subobscura* from temperate regions and *D. melanogaster* from (sub)tropical regions (Kraaijeveld and Godfray 1999). *D. subobscura* is more likely to be exposed to temperatures near 15°C than *D. melanogaster*. One explanation of our results is that 25°C could be a stressful temperature, associated with a high risk of desiccation for *D. subobscura*. Therefore, larvae placed at 25°C should pupate lower - *i.e.* on or near the substrate in relatively moist conditions - than those placed at 15°C. If survival of pupae close to substrate is indeed higher, temperature dependent pupation site selection by *D. subobscura* would be adaptive (Rodriguez et al. 1992). Schnebel and Grossfield (1992) suggested that the lack of upward movement may also help avoid heat stress at high temperatures. The opposite pattern was observed for *D. melanogaster* larvae. Placed at 25°C, a temperature to which they are better adapted than *D. subobscura*, *D. melanogaster* larvae could pupate higher - *i.e.* where conditions are drier - than those placed at 15°C, because of a lower desiccation risk. Pupation height differences may not depend on temperature per se, but rather on correlates of temperature, such as the moisture content of the food or the humidity of the air (Dillon et al. 2009).

The effect of a higher temperature on larval pupation behavior differed between control and parasitized larve. While the increase of pupation height observed in *D. melanogaster* larvae placed at 25°C was weaker for parasitized larvae, the decrease in pupation height of *D. subobscura* larvae was more pronounced in parasitized larvae compared to unparasitized larvae. The longer the pupae develop, the longer the pupae will be exposed to environmental threats. *A. tabida* spends more time within the puparium than its two main hosts, and therefore it is at risk of desiccation or predation for a longer time span (Moreau et al. 2002; Seyahooei et al. 2009). Selection may thus differentially affect hosts and parasititoids. To minimize the risk of desiccation, parasitized larvae of the two species placed at 25°C should pupate more closely to the substrate than unparasitized ones. Our results can also partly be explained by the observations of Seyahooei et al. (2009) who found that larvae parasitized by *A. tabida* travelled less while foraging compared to unparasitized larvae. Moreau et al. (2002) have also shown that parasitism by *A. tabid*a leads to a reduction of host larval activity, due to the venom injected by the ovipositing parasitoid female.

Our results showed that at 25°C, *D. melanogaster* larvae parasitized by *A. tabida* pupated more closely to the substrate than unparasitized ones. These results are consistent with those of Seyahooei et al. (2009) who found that *D. melanogaster* larvae alter their pupation

behavior after attack by *Asobara* parasitoids. They suggested that *Asobara* larvae of several species are able to manipulate the host to obtain a pupation site that minimizes mortality risk close to the substrate. We observed the same pattern for *D. subosbcura* larvae parasitized by *A. tabida*, which also pupated lower than unparasitized larvae at 25°C. As we found that *D. melanogaster* and *D. subobscura* larvae responded differently to temperature, a similar response to parasitism is in support of host manipulation by the parasitoid. Seyahooei et al. (2009) showed that different *Asobara* species alter *D. melanogaster* pupation site selection in a different way. Our results suggest that *A. tabida* may alter the larval pupation behavior of its two main hosts in the same way, by decreasing larvae pupation height. Parasitized and control larvae of both host species showed similar pupation heights when placed at 15°C, indicating that the change in pupation height by *A. tabida* is larger when the risk of desiccation of the pupae is higher at 25°C.

Drosophila larvae that had been exposed to *Asobara tabida* but were not parasitized pupated significantly lower than larvae from the control group (except for *D. melanogaster* larvae placed at 15°C). In *Drosophila melanogaster*, foraging behavior is controlled by the *for* gene, and its alleles induce two behavioral phenotypes, known as 'rovers' and 'sitters' (Seyahooei et al., 2009). Rovers move more during foraging and pupate higher compared to sitters (Graf and Sokolowski, 1989; Sokolowski, 1980). This difference in movements while foraging results in rovers larvae being more attacked by vibrotactic parasitoids such as *A. tabida*, that locate their hosts by reacting to the vibrations they make (Kraaijeveld and van Alphen, 1995). As sitters pupate closer to the medium, the mean pupation height of unparasitized larvae exposed to the parasitoid would be lower than larvae from the control group, just through non-random parasitism rates for different types of larvae. However, the relative proportion of rovers and sitters varies strongly in field populations, depending on food availability and on the different probabilities that both morphs have to be attacked by parasitoids (Sokolowski and Turlings, 1987). This proportion is unknown in our rearings populations, but could clarify our results.

Otherwise, van Alphen and Galis (1983) have shown that two hours exceeds the time needed by an *A. tabida* female to parasitize 20 *D. melanogaster* larvae. It is thus likely that all 20 larvae exposed to a parasitoid female during two hours have been stung at least once, even if no oviposition occurred.

In our experiments, both stung larvae and parasitized larvae differed from control ones in their pupation behavior, indicating that the sting and possibly the oviposition by the female parasitoid alter the host's pupation behavior. The observation that the stinging by an adult

female parasitoid already changes pupation behavior challenges the interpretation of Seyahooei et al. (2009) that the juvenile parasitoid manipulates its host's behavior. Possibly, physical damage associated to the sting reduces larval activity and as a consequence its pupation behavior. However, the stronger effect of parasitism is still in favour of the hypothesis of Seyahooei et al. (2009) that *A. tabida* larvae would manipulate its host behavior, even if we cannot exclude an effect of the venom - injected by the parasitoid female during oviposition (Moreau et al. 2002) - on larvae behavior.

This observation shows that one needs to be careful in concluding that the differences in larvae pupation behavior are associated to the parasitoid larvae manipulating its hosts. Research into the mechanism that causes the change in pupation height is needed before it can be decided if hosts are indeed manipulated, or that the change in pupation height is due to a non-adaptive side effect of parasitism.

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LEGENDS

Table 1 Survival and parasitism rate (%) of larvae of two species of Drosophila (D. mel : *D. melanogaster*; D. sub : *D. subobscura*) under different temperatures and exposure to parasitism by *A. tabida* (control group: unparasitized larvae not exposed to the parasitoid ; experimental group: larvae exposed to the parasitoid).

		Survival rate	Parasitism rate
D. mel 15°C	Control group	91	
	Experimental group	76	88

D.mel 25°C	Control group	98	
	Experimental group	87	39
D. sub 15°C	Control group	68	
	Experimental group	45	93
D. sub 25°C	Control group	72	
	Experimental group	71,5	45

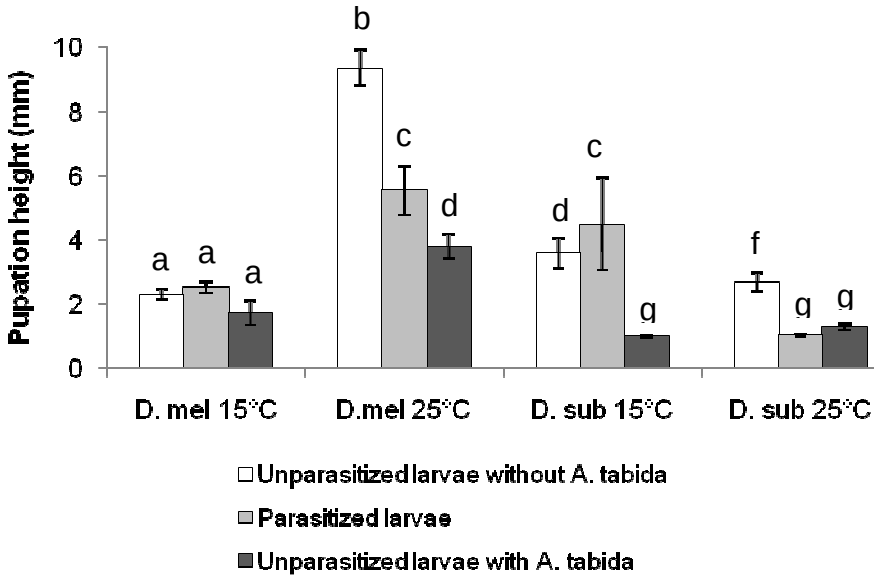

Fig. 1 Mean pupation heights (mm) of larvae of two species of Drosophila (D. mel : *D. melanogaster*; D. sub : *D. subobscura*) under different temperatures and exposure to parasitism by *A. tabida* (unparasitized larvae not exposed to the parasitoid; parasitized larvae

exposed to the parasitoid; unparasitized larvae exposed to the parasitoid). Different letters : $p<0.001$ (Weibull model), error bars : standard errors.

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Résumé

Durant cette thèse, nous avons cherché à (1) déterminer le rôle du climat et de facteurs biotiques associés à celui-ci dans la sélection d'adaptations locales chez des parasitoides de drosophiles (2) comprendre comment la plasticité phénotypique des traits d'histoire de vie a évolué en réponse à l'environnement et aux stratégies de maturation de ces organismes.
Contrairement aux consommateurs primaires pour lesquels la température d'origine affecte directement l'évolution des histoires de vie, il semblerait que les facteurs biotiques dépendants du climat comme la distribution des hôtes et la compétition interspécifique soient le moteur principal de la sélection naturelle chez les parasitoides. La distribution des hôtes expliquerait les très fortes variations géographiques observées sur des échelles fines, comme l'existence de populations proovogéniques ou synovogéniques au sein d'une même espèce, ainsi que de populations capables ou non de lipogenèse à l'age adulte. Cette dernière variation aurait notamment affecté les compromis entre traits et l'évolution du taux de métabolisme. La force de la plasticité phénotypique présente également de fortes variations géographiques. Celles-ci peuvent être attribuées à la variabilité de l'environnement d'origine et non aux histoires de vie des organismes. Prédire l'évolution des parasitoides en réponse au réchauffement global nécessite donc d'intégrer non pas seulement un effet direct du climat, mais également les facteurs biotiques et la variabilité environnementale associés au climat.

Mots clés: Adaptations locales, traits d'histoire de vie, stratégies de reproduction, plasticité phénotypique, normes de réaction, climat, distribution des ressources, compétition, invasions biologiques, taux de métabolisme, parasitoides de drosophiles.

Abstract

In this thesis, we investigated (1) the relative role of climate and biotic factors varying with climate in the selection of local adaptations of drosophila parasitoids (2) how the phenotypic plasticity of life history traits has evolved in reponse to the environment and egg maturation strategies of these organisms.
Contrarily to the primary consumers in which evolution of life histories is directly affected by temperature, biotic factors varying with climate, such as host distribution and interspecific competition may be the principal agent of natural selection in parasitoids. Host distribution may explain the very strong variations that we observed, such as the existence of proovigenic and synovigenic populations in a same species and populations able or not to synthesise lipids during adult life. This last intraspecific variation may have affected trade-offs between traits and the evolution of metabolic rate. Strong variations in the level of phenotypic plasticity were also observed. Variability of the environment of origin explained these variations whereas life histories of organisms did not. To predict evolution of parasitic wasps in response to the global warming, biotic factors and environmental variability depending on climate should thus be integrated, and not only climate.

Key words: Local adaptations, life history traits, reproductive strategies, phenotypic plasticity, reaction norms, climate, resource distribution, competition, biologic invasions, metabolic rate, drosophila parasitoids.