Succès invasif de deux insectes introduits aux Îles Kerguelen : le rôle des ajustements morphologiques et écophysiologiques aux nouvelles conditions environnementales

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présentée par

Mathieu Laparie

Préparée à l’unité de recherche 6553 ÉCOBIO
« Écosystèmes, Biodiversité, Évolution »

Succès invasif de deux insectes introduits aux Îles Kerguelen : le rôle des ajustements morphologiques et écophysiologiques aux nouvelles conditions environnementales

Thèse soutenue à Paimpont
le 9 décembre 2011

devant le jury composé de :

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Succès invasif de deux insectes introduits aux Îles Kerguelen : le rôle des ajustements morphologiques et écophysiologiques aux nouvelles conditions environnementales

Invasive success of two alien insects at the Kerguelen Islands: role of morphological and ecophysiological adjustments to the novel environmental conditions

À mes parents, mon frère et ma sœur,
à Chewb, à toutes les mouches sans ailes.

Cette thèse a été réalisée de 2008 à 2011 à la Station Biologique de Paimpont, au sein de l'équipe PaysaClim « Paysages, Changements climatiques, Biodiversité » du laboratoire Écobio « Écosystèmes, Biodiversité, Évolution » (UMR CNRS 6553, Université de Rennes 1). Ce laboratoire est fédéré par l'OSUR (Observatoire des Sciences de l'Univers de Rennes) et le Caren (Centre Armoricain de Recherches en Environnement).

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PhD. student [ˌpiː eɪtʃ ˈdiː ˈstuːdnt]

Alien species introduced at the Kerguelen Islands (represented in red for ease of visualization). May have big nose, curly hair, and a high tendency to persecute flies, but other phenotypes and behaviors are frequently observed. As illustrated, may alter biotic interactions through predation and/or competition. This species has not established self-sustaining populations yet, and its persistence is only due to repeated introductions. Drawing by Emmanuel Lepage, Port-aux-Français.

REMERCIEMENTS

C'est paradoxalement en écrivant les premières pages de ce manuscrit que je le termine. L'exercice est difficile car voilà que je me retourne sur plusieurs années intenses et riches en rencontres, en émotions, en sacrifices. Les visages fusent dans le désordre le plus total dans mon esprit à la simple écriture de ces premiers mots. Certaines anecdotes particulièrement futiles (et donc intéressantes) me reviennent même brutalement après s'être évanouies dans l'oubli depuis bien longtemps. L'envie de définitivement cristalliser dans ces remerciements tous les souvenirs qui me viennent est forte, mais mon côté *écolohippie* voulant sauver la forêt tropicale de l'industrie du papier m'interdit l'exhaustivité. Ou bien sont-ce l'impatience et la date butoir qui me pressent ?

L'affaire des remerciements est d'autant plus délicate que l'enjeu est grand : ces pages sont à coup sûr celles qui focaliseront l'attention – et c'est d'ailleurs bien normal, c'est ici que se trouvent les résultats les plus croustillants de trois ans de thèse ! S'il existait un *Journal of Acknowledgements*, les *Nature*, *Science* et consorts auraient du souci à se faire (quant à moi, je publierais peut-être davantage). Pourtant, ici, pas une phrase en anglais, pas de méthodologie, pire encore, pas même de bibliographie à mettre en forme. Impensable. J'ai songé à combler les lacunes scientifiques de ces remerciements en leur accordant la rigueur statistique qu'ils méritaient. L'idéal aurait été une analyse en composantes principales sur un échantillon d'individus sauvages collectés dans l'écosystème de cette thèse, puis mesurés selon un ensemble de variables quantitatives reflétant les raisons pour lesquelles je pense à eux en faisant la rétrospective de ces trois dernières années. J'ai toutefois réalisé que cela me vaudrait sans doute les remerciements les plus rasoirs de l'histoire des remerciements, ainsi que la perte de chacun de mes amis, jusqu'au dernier. Dommage, car imaginer la dispersion des individus (notamment les extrêmes) m'amusait déjà ! Rester dans la tradition n'enlève toutefois rien à la sincère émotion qui m'envahit lorsque je pense à tous ceux auxquels je dois la riche expérience qu'a été cette thèse.

Le jury

Je remercie tout d'abord les membres du jury pour le temps qu'ils ont accordé à l'évaluation de mon travail et pour avoir tourné les quelques 200 pages qui suivent celle-ci. *Many thanks* à Kevin Hughes et à Daniel Simberloff pour avoir accepté de participer à ce jury en tant que rapporteurs, ainsi qu'à Vincent Debat et Brent Sinclair pour avoir accepté d'examiner ce manuscrit. J'espère être à la hauteur.

L'encadrement, la formation

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Les proches

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LIST OF PAPERS

This thesis is based on the following articles, which are either published, under review, submitted or in preparation, and are hereafter referred to by their roman numerals:

ARTICLE I	Lebouvier M., **Laparie M.**, Hullé M., Marais A., Cozic Y., Lalouette L., Vernon P., Candresse T., Frenot Y. & Renault D. 2011. The significance of the sub-Antarctic Kerguelen Islands for the assessment of the vulnerability of native communities to climate change, alien insect invasions and plant viruses. *Biological invasions* 13, 1195–1208.
ARTICLE II	**Laparie M.**, Vernon P., Cozic Y., Frenot Y., Renault D.* & Debat V.* When aliens meet novel selection pressures: morphological change of the wing in a flying insect invading a world of flightlessness. *In prep.* (* for equal contributions of authors)
ARTICLE III	**Laparie M.**, Lebouvier M., Lalouette L. & Renault D. 2010. Variation of morphometric traits in populations of an invasive carabid predator (*Merizodus soledadinus*) within a sub-Antarctic island. *Biological Invasions* 12, 3405–3417.
ARTICLE IV	**Laparie M.**, Renault D., Lebouvier M. & Delattre T. Is dispersal promoted during range expansion of invasive species? A synchronic approach using morphometrics in a ground beetle invading sub-Antarctic islands. *Submitted to Biological Invasions.*
ARTICLE V	Hidalgo K., **Laparie M.**, Bical R., Siaussat D., Larvor V. & Renault D. Metabolic fingerprinting and molecular responses to salinity in the invasive ground beetle *Merizodus soledadinus* at the Kerguelen Islands. *Submitted to Journal of Insect Physiology.*
ARTICLE VI	**Laparie M.**, Larvor V., Frenot Y. & Renault D. 2011. Starvation resistance and effects of diet on energy reserves in a predatory ground beetle (*Merizodus soledadinus*; Carabidae) invading the Kerguelen Islands*. Comparative Biochemistry and Physiology Part A*, DOI 10.1016/j.cbpa.2011.09.011.
ARTICLE VII	**Laparie M.**, Bical R., Larvor V., Frenot Y. & Renault D. Habitat phenotyping of two sub-Antarctic flies by metabolic fingerprinting: evidence for a species outside its home? *Submitted to Metabolomics.*

For ease of reading, the references cited in these manuscripts were all grouped in a single section at the end of this thesis.

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Outline

AVANT-PROPOS

La variété des terminologies employées en biologie des invasions (synonymes pour des concepts identiques, définitions multiples d'un même terme, *etc.*) a longtemps constitué l'un des principaux freins à l'établissement de généralisations conceptuelles robustes (Davis & Thompson 2000, Richardson et al. 2000, Huey et al. 2005, Falk-Petersen et al. 2006, Lockwood et al. 2007, Blackburn et al. 2011). Certains auteurs se sont attachés à définir une base terminologique commune, aussi nous adopterons dans le présent manuscrit les définitions recommandées par Falk-Petersen et al. (2006), synthétisées sur la base de dictionnaires biologiques, traités internationaux et articles scientifiques publiés au cours des 30 dernières années :

- espèce native (*native species*) : espèce présente dans son aire naturelle, ancienne ou actuelle, et dans les limites de son potentiel de dispersion (*i.e.*, organisme dont la dispersion est indépendante de l'intervention humaine) ;
- espèce introduite (*alien species*) : espèce présente en dehors de son aire naturelle, ancienne ou actuelle, et au-delà des limites de son potentiel de dispersion, incluant toute partie de l'organisme susceptible de survivre et de se reproduire (*i.e.*, organisme dont la dispersion est provoquée par l'action humaine) ;
- espèce naturalisée (*naturalized species*) : espèce introduite ayant constitué une population stable (auto-entretenue) ;
- espèce invasive (*invasive species*) : espèce introduite s'étant établie dans une nouvelle zone (*i.e.*, naturalisation) et dont la répartition est en expansion ;
- introduction (*introduction*) : mouvement direct ou indirect, par action humaine, d'un organisme depuis son aire native, ancienne ou actuelle, vers une zone en dehors de son potentiel de distribution.

CHAPITRE 1

INTRODUCTION GÉNÉRALE

I do not deny that there are many and grave difficulties in understanding how several of the inhabitants of the more remote islands, whether still retaining the same specific form or modified since their arrival, could have reached their present home.

— Charles Robert Darwin, 152 years ago (*On the origin of species*, 1859)

1.1. 10 000 ans d'invasions biologiques liées à l'Homme, 30 ans de biologie des invasions

La structure des biomes du globe est naturellement en déséquilibre permanent du fait des extinctions qui réduisent la diversité spécifique, de la spéciation et de la différenciation qui l'augmentent, ou encore de l'homogénéisation liée à la dispersion des organismes (Brown & Sax 2004). La biodiversité est ainsi fondamentalement dynamique dans le temps et l'espace, si bien qu'il existe de nombreuses traces fossiles d'épisodes d'expansion géographique ou d'extinction d'espèces. Les environnements ont de tout temps été façonnés par de grands évènements de perturbation, tels que la dérive et la collision des continents, l'ouverture et la fermeture d'océans, les variations du niveau de la mer, ou encore les cycles glaciaires. Ces vastes changements ont mis certaines espèces en contact avec des milieux jusqu'alors au delà de leurs capacités de dispersion, dans lesquels elles ont pu rencontrer un fort succès au point de les envahir.

La colonisation naturelle de zones isolées par les barrières biogéographiques peut également intervenir de manière sporadique, notamment à travers la dispersion passive par le vent, les courants marins, les radeaux flottants ou encore la zoochorie (Barnes et al. 2006). Bien que ces évènements indépendants soient singuliers et difficilement remarquables, leur nombre et leurs conséquences sur la biodiversité sont considérables à l'échelle de l'histoire de la vie (Brown & Sax 2004). Ces auteurs rappellent ainsi que les organismes qui peuplent les milieux terrestres des îles océaniques descendent exclusivement d'envahisseurs, ces îles étant des volcans isolés qui n'ont jamais été connectés aux autres milieux terrestres.

Le rythme et l'ampleur des invasions biologiques ont néanmoins considérablement augmenté depuis l'expansion de notre propre espèce (Cassey et al. 2005) – dont la présence initialement limitée au continent africain s'est rapidement étendue à la plupart des terres de la planète. Les impacts de l'Homme moderne sur son environnement (changements climatiques, exterminations d'espèces, mondialisation et connexion de zones précédemment isolées, transformation des habitats, transports ; Brown & Sax 2004) modifient en effet profondément le régime de répartition des espèces et les assemblages écologiques, depuis l'échelle locale jusqu'à l'échelle globale (Cassey et al. 2005). Cette rupture dans la dynamique de changement a donné lieu à une séparation fondamentale entre les invasions faisant suite à des colonisations naturelles et celles d'origine anthropique (Cassey et al. 2005). L'usage du terme « invasif » est généralement réservé aux organismes initialement introduits par intervention humaine (Falk-Petersen et al. 2006 ; **partie Avant-propos**), tandis que les changements naturels de la structure et de la composition spécifique des communautés correspondent à des « successions » (Chapman & Reiss 1999). Malgré la controverse autour du rôle à accorder aux scientifiques dans la gestion et la régulation des invasions (*cf.* Brown & Sax 2004, Brown & Sax 2005, Cassey et al. 2005), cette dichotomie entre phénomènes naturels et anthropiques est largement reconnue. Ce travail de thèse ayant pour objet les invasions biologiques d'origine anthropique, toute mention des invasions dans la suite de ce manuscrit fera référence aux cas associés à l'activité humaine (définition de Falk-Petersen et al. 2006).

Bien que la période néolithique marque le début des invasions biologiques d'origine anthropique selon certains auteurs (Webb 1985, Heywood 1989), la biologie des invasions en tant que discipline de recherche n'a pris son essor que depuis la fin du XX^{ème} siècle (Fig. 1). La publication en 1958 de l'ouvrage phare d'Elton, intitulé *The ecology of invasions by animals and plants*, est souvent considérée comme l'origine de la discipline (Richardson & Pyšek 2008, Davis 2009). Toutefois, selon Simberloff (2011), la biologie des invasions moderne est trop récente pour avoir été fondée par cette monographie : les premières études n'ont été publiées que suite au programme *Ecology of biological invasions* initié en 1982 par Harold Mooney et supporté par le *Scientific Committee on Problems of the Environment* (SCOPE). Bien que le travail d'Elton apporte maintenant de nombreux éléments à ce qu'est devenue la biologie des invasions, Simberloff (2011) montre qu'il est trop ancien pour avoir été le moteur du développement de la discipline et qu'il n'a pas influencé la genèse du programme SCOPE porté par Mooney. Ainsi, depuis la fin des années 1980, la littérature sur les invasions biologiques connaît un développement exponentiel (Blackburn et al. 2011, Gurevitch et al. 2011 ; Fig. 1). Cette expansion historique est telle qu'elle a conduit, depuis 1999, à la publication de *Biological Invasions*, une revue scientifique entièrement consacrée à cette thématique aux conséquences écologiques des invasions (Falk-Petersen et al. 2006). L'explosion de cette discipline de recherche a généré de nombreux concepts (Falk-Petersen et al. 2006) et a contribué à la meilleure compréhension du processus d'invasion (Cadotte et al. 2006, Lockwood et al. 2007, Blackburn et al. 2009, Davis 2009, Blackburn et al. 2011).

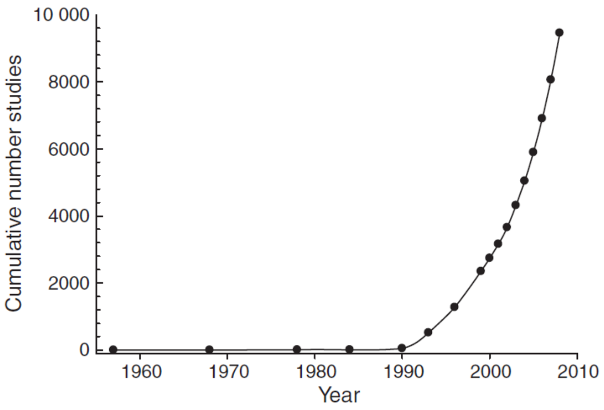

Fig. 1. Nombre cumulé d'études publiées en écologie des invasions de 1958 à 2008, d'après Gurevitch et al. (2011). Recherche bibliométrique Web of Science selon la formule : "inva*" and "(ecol* or plant or invert*)", après élimination des thématiques non liées à l'écologie (ingénierie, oncologie, *etc.*). L'intervalle de temps est affiné à mesure que le nombre d'études publiées croît, de 10 ans jusqu'à un an à partir de 2000.

Composantes essentielles des changements globaux, les invasions biologiques sont aujourd'hui considérées comme la seconde cause mondiale d'érosion de la biodiversité (Vitousek et al. 1996, 1997). Elles peuvent altérer les processus écosystémiques (cycles des nutriments, de production, ou régimes de perturbations), ou avoir des conséquences sanitaires et économiques négatives, notamment à travers leurs effets néfastes sur l'agriculture (Mack et al. 2000, Ruiz et al. 2000, Pimentel et al. 2001, Allendorf & Lundquist 2003, Novak 2007, Sax et al. 2007). De nombreux auteurs se sont donc concentrés sur les conséquences des invasions biologiques à l'échelle écosystémique (Novak 2007), mais leur compréhension, leur prévention ou encore la prédiction des risques constituent également des thèmes pivots (Kolar & Lodge 2001) à l'interface entre la biologie des invasions et la biologie de la conservation (*cf.* Ruiz & Carlton 2003). L'explosion rapide de cette discipline et les différentes problématiques auxquelles elle a trait ont cependant conduit à une certaine confusion terminologique (**partie Avant-propos**), voire conceptuelle, dont l'effort de résolution reste récent (Falk-Petersen et al. 2006).

1.2. Théorie(s) synthétique(s) de l'invasion

1.2.1. Vers un cadre conceptuel unique

Gurevitch et al. (2011) indiquent que de réels progrès ont été faits ces dernières années pour définir le cadre conceptuel et synthétique de la biologie des invasions. Cet effort passe par l'intégration des multiples hypothèses relatives aux invasions (*cf.* revues dans Inderjit et al. 2005, Catford et al. 2009) au sein même des principes de base de l'écologie et de la biologie évolutive. Ainsi, Gurevitch et al. (2011) recommandent l'explication des invasions biologiques à travers les processus écologiques et évolutifs généraux que sont la dynamique des populations, la compétition, la prédation, la sélection naturelle, ou encore l'écologie du paysage, plutôt que selon des phénomènes propres et spécifiques à l'invasion. Cette démarche permet notamment une meilleure prise en compte des interactions entre les mécanismes contribuant aux invasions, mais également de la diversité des échelles à considérer pour construire une base théorique synthétique. Le processus d'invasion se décompose en effet en étapes successives, chacune d'entre elles pouvant s'appliquer à des échelles temporelles et géographiques distinctes (Melbourne et al. 2007, Milbau et al. 2009, Gurevitch et al. 2011).

Williamson & Fitter (1996) décrivent en effet l'invasion comme une série de stades à travers lesquels une espèce doit passer pour devenir invasive. Cette trame en stades successifs est largement adoptée dans les communautés de chercheurs en écologie animale selon Blackburn et al.

(2011). Il existe cependant de multiples réinterprétations de cette série puisque certains auteurs la déclinent en trois (arrivée, établissement, dispersion ; Williamson 1996, Freckleton et al. 2006), quatre (arrivée, libération ou fuite, naturalisation, dispersion ; Duncan et al. 2003), ou six étapes (introduction, établissement, naturalisation, dispersion, distribution des populations, dispersion invasive ; Henderson et al. 2006). Par ailleurs, la dimension taxonomique peut également contribuer à subdiviser les approches théoriques du processus d'invasion (Blackburn et al. 2011). Ces auteurs indiquent en effet que la plupart des botanistes adoptent le processus théorique proposé par Richardson et al. (2000), selon lequel les espèces rencontrent une série de barrières abiotiques et biotiques avant de devenir invasives. Les deux approches proposées par Williamson & Fitter (1996) et Richardson et al. (2000) partagent néanmoins leurs concepts fondamentaux et peuvent être fusionnées en dépit de leurs particularités, celles-ci étant essentiellement relatives aux taxons considérés et non au cadre théorique du processus d'invasion. Blackburn et al. (2011) ont proposé une unification des deux visions du processus d'invasion, permettant ainsi de définir le socle commun aux différentes études de cas pour faciliter leur comparaison et leur compréhension.

Le modèle synthétique proposé par Blackburn et al. (2011) décompose l'introduction en deux sous-étapes : le transport, puis la libération (ou fuite) de l'organisme transporté dans le nouvel environnement. Cependant, la libération (ou fuite) des propagules est difficilement applicable aux organismes introduits accidentellement, dont le prélèvement et le transport ne sont pas intentionnels, tels que la majorité des arthropodes terrestres introduits (Hulme et al. 2008, Blackburn et al. 2011). La libération (ou fuite) est par exemple reconnue par Duncan et al. (2003), mais il est important de noter que ces auteurs se sont focalisés sur les oiseaux, des organismes essentiellement transportés pour des raisons récréatives et gardés en captivité (parcs, animaux de compagnie). Blackburn et al. (2011) décrivent ainsi eux-mêmes la libération (ou fuite) de l'organisme transporté comme facultative dans la phase d'introduction. Le transport des propagules dans le nouvel environnement, ainsi que leur prélèvement préalable dans l'aire native (Lockwood et al. 2005), sont quant à eux les éléments essentiels de la phase d'introduction puisqu'ils conditionnent le pool d'organismes qui rencontreront effectivement le milieu cible. Nous identifierons ainsi trois phases centrales que sont **(i) l'introduction, qui inclue le transport des propagules jusqu'au milieu cible, (ii) la naturalisation** et **(iii) la propagation (ou invasion), qui inclue la prolifération et l'expansion géographique**, sur lesquelles peuvent se greffer d'autres phases périphériques parfois considérées selon les besoins des études.

La déclinaison du processus en de telles phases séquentielles suggère une progression discrète de l'invasion, mais celle-ci correspond à l'inverse à un phénomène continu (Richardson et al.

2000, Daehler 2006, Davis 2009). En effet, l'entrée dans une phase n'implique pas la fin des phases précédentes et, à l'inverse, l'invasion doit être perçue comme un ensemble de cycles d'itérations des différentes phases. Ainsi, lorsqu'une espèce introduite commence à se propager et à devenir invasive, les phases d'introduction et de naturalisation peuvent indépendamment perdurer. Selon Davis (2009), cette vision cyclique repose fondamentalement sur la dispersion et la survie jusqu'à l'âge de reproduction, qui dépendent des performances individuelles, la propagation pouvant être considérée comme une propriété relative à la population, issue des deux processus individu-centrés.

1.2.2. La phase d'introduction

L'introduction au sens retenu dans ce travail est indissociable de l'activité humaine et, de ce fait, se distingue du phénomène de colonisation naturelle (Falk-Petersen et al. 2006). Elle repose sur le transport préalable, volontaire ou non, de propagules (graines, œufs, larves, individus matures ; Novak 2007) depuis leur aire native vers une nouvelle zone géographique. Ce voyage constitue le premier obstacle rencontré par les espèces, car la plupart n'y survivent pas (Davis 2009). La pression de propagules est la combinaison de la quantité de propagules par transport et de la fréquence des transports (Lockwood et al. 2005).

1.2.2.1. La pression de propagules

Les pressions de propagules ont été largement intensifiées par la mondialisation et le développement de grands axes d'échanges pour le commerce international (transport maritimes, aériens, routiers, tourisme, *etc.* ; Lockwood et al. 2005, Meyerson & Mooney 2007, Lee & Chown 2009). Les causes d'introduction sont diverses et vont des importations volontaires aux activités logistiques et techniques, incluant même les activités scientifiques (Davis 2009) et les espèces introduites qui peuvent elles-mêmes constituer des vecteurs pour leurs ennemis naturels (*cf.* l'exemple des pucerons introduits et des virus de plantes ; **ARTICLE I**). Les eaux de ballast sont considérées comme un vecteur d'introduction majeur, transportant continuellement des milliers d'organismes marins à travers les eaux et les ports du globe (Carlton 1999, Verling et al. 2005). Les migrations humaines s'accompagnent également d'introductions d'espèces, comme l'ont montré Jeschke & Strayer (2005) en reconstituant l'évolution temporelle des flux d'introductions de vertébrés entre l'Amérique du Nord et l'Europe (Fig. 2).

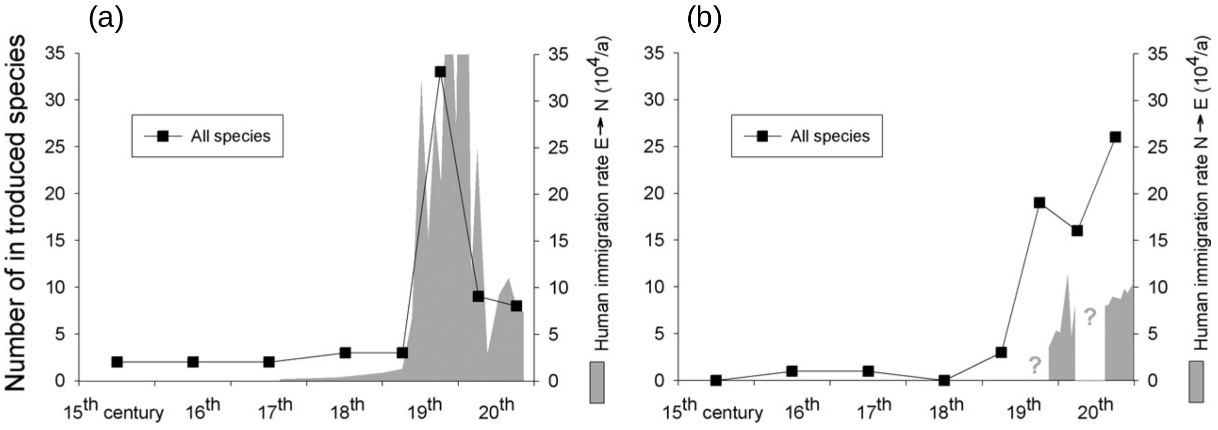

Fig. 2. Evolutions temporelles des introductions de vertébrés de l'Europe vers l'Amérique du Nord (a) et de l'Amérique du Nord vers l'Europe (b), d'après Jeschke & Strayer (2005). Les données de migration humaine sont issues de la littérature (*cf.* Jeschke & Strayer 2005). Les points d'interrogation indiquent les données manquantes.

1.2.2.2. Vecteurs de propagules

Les pressions de propagules propres à chaque voie et chaque vecteur de transport restent néanmoins difficilement quantifiables et, bien que leur nombre soit en augmentation, peu d'études se sont à ce jour focalisées sur cette étape fondamentale de l'invasion (Puth & Post 2005, Lee & Chown 2009). Le nombre d'espèces introduites a fréquemment été utilisé comme témoin indirect de la quantité de propagules propre à chaque voie d'introduction, permettant ainsi d'évaluer les risques. La base de données du réseau NOBANIS (North European and Baltic Network on Invasive Alien Specie) permet ainsi de reconstituer, par vecteur, l'histoire des introductions d'espèces en Europe du Nord. Hulme et al. (2008) ont classé les voies d'introduction selon un système simplifié qui reflète le degré d'intervention humaine (permettant ainsi de mieux cibler les mesures de prévention ; Davis 2009) et ont pu, grâce à ce réseau, comparer leur importance relative. L'exemple des espèces marines illustre la constante augmentation du nombre d'introductions attribuables à chacune de ces voies (Fig. 3). Ces auteurs montrent toutefois que l'importance relative des voies d'introduction évolue différemment selon les taxons considérés. Le nombre d'introductions d'oiseaux, de reptiles et de mammifères par lâchers délibérés a par exemple diminué au cours du 20ème siècle, illustrant à la fois la prise de conscience des problèmes associés aux invasions biologiques de ces taxons et le besoin de renforcer les mesures préventives liées aux autres voies d'introduction (Hulme et al. 2008).

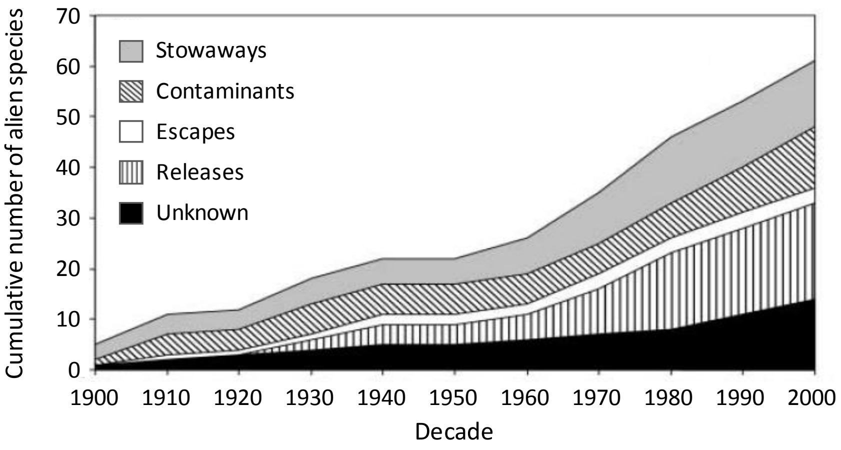

Fig. 3. Evolution temporelle du nombre d'introductions d'espèces marines, par mode d'introduction, en Europe du Nord, d'après Hulme et al. (2008). Données NOBANIS (North European and Baltic Network on Invasive Alien Species, www.nobanis.org).

La nature du vecteur de transport, sa durée, les conditions rencontrées pendant le transport et la qualité des propagules (stade de développement, état biologique) sont autant de facteurs qui conditionnent la survie des propagules et, ainsi, influencent les probabilités de naturalisation dans le nouvel environnement (Davis 2009, Wilson et al. 2009a). Carlton & Ruiz (2005) caractérisent ainsi six unités élémentaires du transport, permettant de mieux comprendre son rôle dans la phase d'introduction et de mieux développer des moyens de gestion et de prévention des risques :

- la *cause du transport*, qui peut être intentionnelle ou non ;
- la *route empruntée*, qui correspond au trajet géographique entre le point de prélèvement et le point d'introduction ;
- le *type de vecteur*, qui peut conditionner l'environnement physique et donc la survie des propagules ;
- le *tempo*, qui décrit la dynamique temporelle du vecteur (fréquence, durée, époque) et ainsi influence la survie à la fois lors du transport et lors de l'arrivée dans le milieu cible (saisonnalité) ;
- les *êtres vivants transportés*, notamment leur diversité, leur quantité et leur stade de développement, qui peuvent influencer le succès d'invasion (Lockwood et al. 2005) ;
- la *force du vecteur*, qui traduit la proportion d'invasions réussies dont le vecteur considéré est responsable.

La sélection d'individus au regard de leurs traits de vie peut par ailleurs intervenir lors de cette étape de transport, avant même la libération des organismes dans le milieu de destination (Genton 2005, Novak 2007). Cette hypothèse d'une sélection non aléatoire lors du transport a été vérifiée chez des carabidés introduits depuis l'Europe vers l'Amérique du Nord par le biais de ballasts solides (Lindroth 1957, Carlton 2011). La cause du transport, le type de vecteur et son tempo apparaissent alors comme des caractéristiques fondamentales dans la sélection des traits de vie des organismes qui rencontreront effectivement le milieu cible. En effet, la cause du transport peut, notamment lorsque celui-ci est intentionnel, conduire à la sélection préalable d'individus selon leurs caractéristiques (*e.g.* commerce), alors que le tempo peut impliquer la multiplication des générations avant l'arrivée à destination. En tant qu'étape basale de l'invasion, la plus étroitement liée à l'activité anthropique, l'introduction (notamment les composantes du transport) est souvent considérée comme l'élément clef de la compréhension du succès ou de l'échec de naturalisation des espèces introduites (Kolar & Lodge 2001, Lockwood et al. 2005).

Davis (2009) souligne que la plupart des espèces introduites échouent avant ce stade de naturalisation et que le nombre d'introductions est par conséquent souvent sous-estimé ; les populations naturalisées étant plus faciles à détecter que les propagules. Un meilleur contrôle des invasions passe pourtant prioritairement par la prévention et, ainsi, par un renforcement des mesures de biosécurité ciblées sur le transport d'espèces (Mack et al. 2000, Courchamp et al. 2003, Jeschke & Strayer 2005, Gren 2008, Lee & Chown 2009). La faible compréhension du poids relatif des différents vecteurs – et des pressions de propagules qui leur sont attribuables – ralentit le développement de mesures de contrôle raisonnées (Lee & Chown 2009).

À travers le programme international *Aliens in Antarctica*, Lee & Chown (2009) ont récemment contribué à combler ce manque. Ces auteurs ont en effet comparé les charges en propagules spécifiques aux différents vecteurs dans les zones antarctique et subantarctique (48°S et plus, Nouvelle-Zélande et Amérique du Sud exclues). Cette étude constitue la toute première mesure de la proportion de propagules effectivement transportée dans les nouveaux milieux naturels, par opposition au plus classique comptage des populations naturalisées. Cette étude a ainsi permis de chiffrer les pressions de propagules pour différentes destinations de la région australe, ainsi que d'identifier la provenance possible de ces propagules. Plusieurs espèces réputées invasives ou cosmopolites ont été détectées, tout comme d'autres espèces natives de la région antarctique, montrant ainsi le rôle des flux anthropiques non seulement sur le transport d'espèces exotiques, mais également sur l'homogénéisation au sein même de la région considérée (Lee & Chown 2009, Hughes et al. 2010). Ce travail a par ailleurs mis en évidence la part importante des vêtements et des bagages dans la proportion de propagules effectivement libérées dans le milieu et, ainsi, de suggérer des mesures de biosécurité spécifiques à ce vecteur. Plus récemment, Hughes et al. (2011) ont montré que l'approvisionnement des bases scientifiques de la région antarctique en nourriture fraîche est également l'un des vecteurs majeurs d'introduction d'espèces. Des études complémentaires sont nécessaires pour développer des réponses adéquates aux échanges de propagules intra-île (îles subantarctiques) ou intra-région (Antarctique), qui peuvent notamment contribuer à la dissémination et l'invasion d'espèces déjà naturalisées (Lee & Chown 2009).

Les mesures de prévention et de conservation issues des recherches fédérées par le programme *Aliens in Antarctica* commencent toutefois à être mises en application dans cette zone. En effet, le Comité pour la Protection de l'Environnement (*Committee for Environmental Protection*, CEP) a adopté en 2011 le *Non-native species manual*, une notice sur la prévention des invasions biologiques entre les sites de l'Antarctique, listant les moyens à mettre en œuvre pour préserver la biodiversité locale (CEP 2011).

1.2.3. La phase de naturalisation

1.2.3.1. Rôle des traits d'histoire de vie

Seule une minorité des espèces introduites parvient à persister et à se reproduire dans le nouvel environnement (Mack et al. 2000, Novak 2007). La survie et la reproduction d'un organisme introduit reposent sur quatre conditions distinctes qu'il doit impérativement compléter (Davis 2009) :

- il doit trouver un environnement dont les conditions abiotiques sont satisfaisantes et dans sa gamme de tolérance (température, salinité, *etc.*) ;
- il doit pouvoir accéder aux ressources nécessaires à sa croissance, sa maintenance et sa reproduction ;
- si sa reproduction est sexuée, il doit trouver un partenaire sexuel, ou ses gamètes doivent rencontrer les gamètes du sexe opposé dans le nouvel environnement ;
- enfin, il doit éviter la mortalité précédant l'âge de reproduction.

Le taux de réussite dans chacune de ces tâches est largement défini par les traits de vie de l'organisme ainsi que par sa plasticité phénotypique (*cf.* synthèse dans Davis 2009). Les changements climatiques sont également un facteur facilitant la naturalisation des espèces, parce qu'ils modifient la relation entre les organismes introduits et les contraintes locales, mais aussi parce qu'ils fragilisent les communautés natives (Chapin et al. 2000, Stachowicz et al. 2002, Frenot et al. 2006).

Dietz & Edwards (2006) indiquent que la naturalisation d'un organisme dépend, en premier lieu, de ses traits de vie à l'instant de l'introduction, lui permettant d'exploiter la ressource disponible, puis, en second lieu, de l'adaptation au nouvel environnement. La plasticité phénotypique peut alors agir à un niveau intermédiaire et tamponner l'effet des conditions locales, permettant aux organismes d'ajuster leurs traits de vie aux nouvelles conditions dès leur entrée dans le nouveau milieu. Ces ajustements comprennent la flexibilité de l'allocation énergétique, des formes de croissance, du comportement, ou encore du régime alimentaire et de la sélection des habitats (Davis 2009 ; **ARTICLES V** et **VI**).

1.2.3.2. La phylogénie prédispose-t-elle au succès des espèces introduites ?

La *Darwin's Naturalization Hypothesis* (Darwin 1859) est l'une des premières hypothèses proposées pour l'identification des espèces à fort potentiel invasif. Indirectement liée aux traits d'histoire de vie, elle suggère que les espèces de plantes ont de plus fortes chances de naturalisation en l'absence d'espèces phylogénétiquement proches dans la communauté native (Darwin 1859, Schaefer et al. 2011). Selon cette idée, des espèces ayant une phylogénie commune ont une plus forte probabilité de partager des niches écologiques similaires et, ainsi, d'être sensibles aux mêmes ennemis et/ou d'entrer en compétition pour la ressource et l'habitat. Leur propension à établir des populations viables dans le milieu cible s'en trouve alors réduite.

Cette hypothèse connaît un regain d'intérêt depuis la multiplication des bases de données sur les espèces naturalisées, ainsi que le développement des outils moléculaires, qui permettent de reconstruire les phylogénies. De nombreux auteurs ont ainsi testé la *Darwin's Naturalization Hypothesis* depuis une dizaine d'années (*cf.* par exemple Daehler 2001, Duncan & Williams 2002, Ricciardi & Mottiar 2006, Jiang et al. 2010, Schaefer et al. 2011). Sa validation fait pourtant débat puisque des résultats très contrastés ont été obtenus au cours de ces différentes études. Cette hypothèse a ainsi été vérifiée pour la première fois expérimentalement grâce à des cultures de bactéries au laboratoire (Jiang et al. 2010). Dans cette étude, les espèces proches de celles qui constituaient les communautés résidentes ont établi moins de populations stables que les espèces dissimilaires. Jiang et al. (2010) indiquent toutefois que ce résultat est en grande partie influencé par la grande résistance de l'une des espèces résidentes à l'une des espèces introduites. Ces auteurs rappellent par ailleurs qu'en dépit du lien entre phylogénie et conservation de niche, il existe dans la littérature de nombreux cas d'espèces proches mais ayant des stratégies de vie différentes. Ainsi, les communautés d'espèces phylogénétiquement proches peuvent montrer une plus grande diversité fonctionnelle que celles rassemblant plus de lignées (Prinzing et al. 2008). Une autre étude, ciblée sur les végétaux de l'archipel des Açores, a montré une corrélation négative entre succès des espèces introduites et proximité phylogénétique (Schaefer et al. 2011). Toutefois, ce travail montre que certains traits écologiques, tels que la forme de vie (annuelles *versus* pérennes) ou la taille des graines constituent de meilleurs prédicteurs du succès des espèces introduites (Schaefer et al. 2011).

À l'inverse, il existe dans la littérature de multiples exemples de corrélations positives entre proximité phylogénétique et succès des espèces introduites (*cf.* par exemple Simberloff 1986, Daehler 2001; Duncan & Williams 2002). Ces résultats sont en désaccord avec l'hypothèse darwinienne précédente. Ils correspondent à une alternative que Darwin avait lui-même initialement envisagée : la *pre-adaptation hypothesis*, selon laquelle les espèces proches des espèces natives peuvent être pré-adaptées au milieu cible (mutualisme avec espèces autochtones, compatibilité avec les contraintes abiotiques) et, ainsi, mieux s'y développer que des espèces dissimilaires (Darwin 1859, Daehler 2001, Ricciardi & Mottiar 2006, Davis 2009).

Le travail de Ricciardi & Mottiar (2006) réalisé sur des espèces de poissons dans différentes zones géographiques tend à invalider les deux hypothèses darwiniennes. Il est toutefois important de préciser que les introductions de poissons ont, pour une part importante, été volontaires (récréation, élevages, *etc.*). Tel que déjà démontré chez les oiseaux (Lockwood 1999), les taxons préférés par l'Homme ont plus de chances d'être introduits dans le nouvel environnement, biaisant par conséquent la relation possible entre phylogénie et proportion d'espèces effectivement naturalisées.

Sax & Brown (2000) définissent le succès des espèces introduites comme un faux paradoxe. En effet, les espèces introduites sont communément vues comme des espèces naïves dans leur milieu cible, leur succès aux dépens des espèces natives (dont l'histoire évolutive est associée à ces milieux) étant alors contre-intuitif. Toutefois, une large part d'entre elles sont en réalité pré-adaptées à leur nouvel environnement (Sax & Brown 2000). De plus, il est important de remarquer que de nombreuses espèces introduites constituent leurs premières populations stables dans des habitats perturbés par l'Homme, telles que les espèces rudérales (Sax & Brown 2000). Ces espèces sont adaptées aux habitats anthropisés et perturbés, que ce soit dans le nouvel environnement ou dans leur aire d'origine, à l'inverse des espèces résidentes (Sax & Brown 2000). En conclusion, la relation entre la phylogénie des espèces et leur chances de succès dans un nouvel environnement apparaît difficilement généralisable. Elle est dépendante des modèles, des régions, du niveau de perturbation et de l'historique des introductions dans les zones considérées. Selon Williams (1951), les avantages de la proximité entre espèces introduites et espèces résidentes pourraient contrebalancer les inconvénients. D'une manière générale, la phylogénie n'est vue dans les hypothèses de Darwin que comme un prédicteur des traits de vie des espèces et de leur niche écologique, eux-mêmes prédicteurs du succès des invasions. Cette approximation doit être considérée avec précaution car les phénomènes de convergence et de divergence évolutives peuvent la confondre (Davis 2009).

1.2.3.3. Invasibilité du milieu cible

Les hypothèses darwiniennes sur la naturalisation impliquent que le succès des espèces introduites est étroitement lié aux spécificités du milieu receveur. La sensibilité d'un milieu à la colonisation et à la naturalisation de nouvelles espèces est définie par son invasibilité ; celle-ci est variable dans le temps et dépend à la fois de caractéristiques biotiques et abiotiques (Davis et al. 2000, Davis 2009). L'invasibilité a ainsi fréquemment été associée à la diversité des communautés résidentes, c'est-à-dire leur diversité spécifique ou leur diversité fonctionnelle, la variation de disponibilité des ressources, les stress physiques, ainsi que les ennemis ou espèces mutualistes des espèces introduites (Davis 2009).

Selon Williamson (1996), l'adéquation entre les préférences d'une espèce et les conditions physiques de son nouvel environnement est *a priori* un prédicteur fiable de la vulnérabilité du milieu face à cette espèce (*i.e.* du risque d'invasion par cette espèce). À titre d'exemple, les eaux froides de l'Océan Antarctique ont par le passé constitué une barrière physique à la naturalisation des espèces introduites mais les changements climatiques, particulièrement intenses dans cette zone, abaissent progressivement cette barrière et augmentent l'invasibilité des eaux antarctiques (Aronson et al. 2007). Comprendre et prédire l'effet des changements climatiques sur de telles barrières physiques est un enjeu crucial de la biologie des invasions (**ARTICLE I**). En effet, les changements climatiques sont un facteur externe qui n'est pas directement spécifique des propriétés intrinsèques des environnements, il peut donc influencer de manière simultanée l'invasibilité de tout une gamme d'environnements d'une région donnée du globe. Les modèles de prédiction basés sur les enveloppes climatiques des espèces dans leur zone native doivent cependant être interprétés avec précaution. En effet, le climat n'est pas le seul facteur limitant la distribution des espèces, l'enveloppe climatique naturelle peut ainsi être non représentative de la gamme de tolérance réelle (Sax et al. 2007).

La résistance biotique d'un environnement aux invasions (autrement dit, les paramètres biotiques réduisant son invasibilité) relève de la théorie des niches. En effet, les communautés saturées (Smith & Shurin 2006) offrent moins de niches vacantes et de ressources disponibles pour de nouvelles espèces que des communautés paucispécifiques, ce qui leur confère ainsi une plus faible invasibilité. Cette hypothèse correspond à la *Diversity-Invasibility Hypothesis* d'Elton (1958), également suggérée par Darwin dans *On the origin of species* (1859). Elle comprend les relations biotiques horizontales (compétition) et verticales ou *top-down* (prédation, parasitisme, *etc.*, *cf. Ennemy Release Hypothesis* ; Crawley 1987, Williamson 1996).

Cette hypothèse basée sur la diversité est cependant vivement controversée en raison de résultats souvent contradictoires dans les études en conditions naturelles et/ou à grande échelle (*cf.* revue dans Davis 2009). Les invasions biologiques ont par ailleurs conduit à l'augmentation de la diversité dans de nombreuses communautés et régions du monde, prouvant ainsi que peu d'environnements sont saturés en espèces et que la plupart sont théoriquement invasibles dans une certaine mesure (Williamson 1996, Sax et al. 2007, Davis 2009). La *Diversity-Invasibility Hypothesis* doit alors englober d'autres facteurs : elle repose notamment sur la condition de dépendance entre la diversité des communautés résidentes et la disponibilité des ressources limitantes (Fridley et al. 2007), cette relation n'étant toutefois pas systématique dans la nature.

Huston & DeAngelis (1994) sont les premiers à avoir mis en lumière la relation entre les variations de la disponibilité des ressources et l'invasibilité d'un milieu : si l'hétérogénéïté spatiale et temporelle de la quantité de ressources les rend au moins temporairement non limitantes, alors les espèces introduites ont une probabilité accrue de persister dans le nouvel environnement. Ainsi, Davis et al. (2000) proposent que les augmentations transitoires de la quantité de ressources (*pulses*) augmentent l'invasibilité d'un environnement. Cette théorie a été confirmée à de multiples échelles, contrairement à l'hypothèse fondée sur la diversité des communautés résidentes (Jiang & Morin 2004, Stachowicz & Byrnes 2006, Williams & Smith 2007, Yang et al. 2008). Elle constitue donc, à ce jour, le prédicteur le plus fiable de l'invasibilité d'un milieu selon Davis (2009).

1.2.4. La phase de propagation, ou invasion

La propagation (ou étape d'invasion *stricto sensu*) d'une espèce naturalisée est la dernière condition du processus d'invasion : l'aire de répartition s'étend dans le nouvel environnement par croissance et multiplication des foyers populationnels à partir de la population initiale (Blackburn et al. 2011). Elle relève selon Falk-Petersen et al. (2006) d'un processus de colonisation propre aux espèces introduites et requiert de nouveaux épisodes de naturalisation. En effet, les individus qui parviennent à passer la barrière environnementale à la dispersion doivent à leur tour constituer des populations stables pour contribuer à l'expansion de l'espèce dans la zone non-native (Reise et al. 2006, Blackburn et al. 2011). L'invasion peut également opérer de manière plus progressive dans l'espace, par l'unique expansion géographique de la population initiale, sans étapes saltatoires de migration.

1.2.4.1. Une répétition d'évènements de naturalisation

Le franchissement de la barrière environnementale à la dispersion (Blackburn et al. 2011) peut intervenir lorsque la population initiale croît et engendre des individus migrants (*cf.* causes ultimes et proximales, Bowler & Benton 2005). Ce phénomène de migration se répète inévitablement dans le temps tant que la population source se maintient, tandis que l'étape d'introduction dans le nouvel environnement peut, dans de rares cas, avoir été unique. Ainsi, une fois une espèce naturalisée hors de son aire native, la pression de propagules vers les secteurs adjacents s'accroit exponentiellement à mesure que de nouvelles populations stables sont fondées. La colonisation de nouveaux sites à l'échelle régionale peut parallèlement relever d'autres vecteurs naturels tels que l'eau, le vent et les animaux, ou être provoquée par de nouvelles introductions d'origine anthropique (Lee & Chown 2009). Dans ce dernier cas, l'organisme introduit est prélevé accidentellement ou non dans une population naturalisée, puis transporté dans un nouveau secteur.

Selon Reise et al. (2006), les individus introduits depuis leur zone native et ceux issus du point d'introduction sont *a priori* sujets aux mêmes pressions lorsqu'ils colonisent un nouveau secteur et y fondent une population stable. Cette vision est en accord avec l'idée de Davis (2009) selon laquelle la troisième étape du processus d'invasion, la propagation, est la conséquence à l'échelle populationnelle de la survie et de la reproduction des individus. Les concepts précédemment détaillés pour l'étape de naturalisation (**partie 1.2.3.**) peuvent alors être appliqués à la propagation. Cette étape ultime étant analogue à une multiplication des évènements de naturalisation, les traits de vie des individus en conditionnent, de la même manière, le succès. Ainsi, l'apport de nouveaux génotypes à travers le maintien des flux de propagules peut également être un déclencheur de l'étape d'invasion d'une espèce (Simberloff 2009a). Largement étudiée chez les espèces végétales,la relation entre les la cette étape et les traits de vie s'illustre par exemple par une forte capacité à envahir le nouvel environnement chez les espèces dont le potentiel reproducteur et/ou disperseur est élevé (Rejmánek et al. 2005, Pyšek & Richardson 2007, Schaefer et al. 2011 ; **ARTICLE IV**). Ces caractéristiques doivent être reliées aux stratégies adaptatives des espèces. Sakai et al. (2001) rappellent ainsi que quel que soit leur taxon, les espèces à fort succès colonisateur ont généralement en commun des histoires de vie caractéristiques d'une sélection de type *r*, soit un temps de génération faible, une forte fécondité et un fort taux de croissance, ainsi que la capacité à basculer entre les stratégies de type *r* et *K*. Kolar & Lodge (2001) soulignent cependant la nécessité de davantage vérifier ces idées de manière quantitative, tandis que Lawton et al. (1986) montrent en combinant des études théoriques et empiriques que la stratégie *r* n'est pas un élément déterminant du succès des invasions. L'effet facilitateur de la plasticité phénotypique a quant à lui souvent été confirmé (Richards et al. 2006, Wilson et al. 2009b, Davidson et al. 2011).

1.2.4.2. L'évolution et la plasticité phénotypique au cœur du succès des invasions

Le succès d’une invasion dépend des caractéristiques du couple entre l’espèce introduite et l’écosystème cible, non de leurs propriétés intrinsèques propres (Shea & Chesson 2002). Il repose sur l’adéquation entre les traits de vie de l’envahisseur et les propriétés du milieu envahi. Selon Facon et al. (2006), trois scénarios peuvent expliquer l’adéquation d’une espèce à un environnement pourtant déconnecté de son histoire évolutive (Fig. 4). D’abord, une espèce invasive peut être pré-adaptée au milieu dans lequel elle a été introduite, son invasion n’ayant été bloquée jusqu’alors que par ses capacités migratoires, trop faibles pour permettre la colonisation du milieu sans assistance humaine (Fig. 4a). Dans ce cas, l’écosystème natif de l’espèce, lié à son histoire évolutive, est similaire à l’écosystème envahi et a conduit à la sélection de phénotypes adaptés par le jeu des pressions environnementales. Le second cas (Fig. 4b) décrit un changement écologique du milieu cible (changements climatiques, perturbations, destruction d’habitat, altération des communautés, *etc.*), dont les nouvelles propriétés coïncident avec les traits de vie de l’espèce précédemment introduite, augmentant sa fitness et permettant son expansion rapide. Enfin, le changement peut intervenir chez l’espèce, après son introduction, impliquant des phénomènes évolutifs jusqu’à ce que son phénotype et sa stratégie d’histoires de vie rencontrent étroitement les propriétés du milieu cible, favorisant alors la propagation (Fig. 4c). Des exemples *in natura* d'adaptations morphologiques locales, incluant leurs conséquences sur les traits de vie, existent ainsi chez des populations invasives de drosophiles et de saumons (Huey et al. 2005). Les trois scénarios théoriques sont simplifiés aux situations extrêmes mais sont non exclusifs et se combinent dans la nature (Facon et al. 2006).

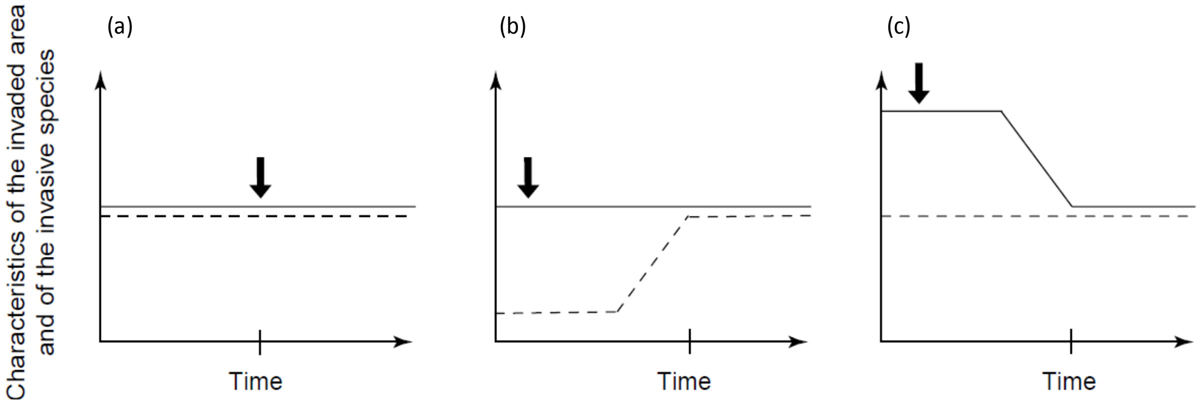

Fig. 4. Scénarios théoriques d'invasion, d'après Facon et al. (2006). Les caractéristiques de la zone envahie sont illustrées par la ligne pointillée, celles de l'espèce invasive par la ligne continue. L'introduction de l'espèce est symbolisée par une flèche, le début de son invasion par une marque en abscisse. Seul un changement du régime de migration est nécessaire pour initier l'invasion (a) ; l'invasion débute après modification du milieu cible, dont les caractéristiques correspondent alors avec celles de l'espèce introduite (b) ; l'invasion suit des changements génétiques chez l'espèce introduite (c).

En accord avec ce dernier scénario, une espèce ne peut devenir invasive que lorsqu'elle est déjà naturalisée dans le nouvel environnement, ce qui implique la reproduction et la multiplication des générations sous les nouvelles conditions environnementales (Blackburn et al. 2011). Ses traits de vie et sa plasticité phénotypique sont ainsi amenés à évoluer de manière intergénérationnelle à travers des processus tels que la sélection naturelle (Schlichting 1986, West-Eberhard 1989, Crooks 2005, Dietz & Edwards 2006, Richards et al. 2006). Ces phénomènes opèrent à différentes échelles temporelles et modifient l'interaction entre la stratégie d'histoires de vie et l'environnement. À ce titre, les changements intervenant *a posteriori* dans le milieu cible sont déterminants dans le succès d'invasion d'une espèce (Dietz & Edwards 2006, Facon et al. 2006, Davis 2009 ; **ARTICLES II, III** et **IV**).

Comme pour l'étape de naturalisation, la plasticité phénotypique est un élément fondamental du succès durable de certaines espèces invasives dans leur nouvel environnement (Richards et al. 2006, Wilson et al. 2009b), dont les conditions sont différentes de celles de son environnement natif et sont de plus progressivement altérées par l'invasion elle-même. Roderick & Vernon (2009) définissent la plasticité phénotypique comme un bouclier contre la sélection naturelle. Elle a été caractérisée comme un phénomène permettant de tamponner et compenser la diversité génétique réduite des espèces introduites dans de nouveaux milieux (Wilson et al. 2009b). Bien qu'elles s'expriment en fonction des variations environnementales, son amplitude, son héritabilité et la qualité des normes de réaction dépendent en premier lieu de la base génotypique existant dans la population (Richards et al. 2006). La plasticité phénotypique constitue de ce fait une caractéristique propre à l'organisme, représentant une stratégie adaptative aux variations de l'environnement

(Gabriel 2005, Trussell & Smith 2000) et potentiellement sujette à la sélection naturelle (Schlichting 1986, West-Eberhard 1989, Dietz & Edwards 2006, Richards et al. 2006). La gamme de tolérance des espèces est ainsi en partie gouvernée par leur plasticité phénotypique, large chez les espèces euryèces et réduite chez les sténoèces. Richards et al. (2006) ont proposé plusieurs scénarios dans lesquels le succès d'une espèce invasive par rapport aux autres peut dépendre de la plasticité phénotypique et de ses conséquences sur la fitness (Fig. 5).

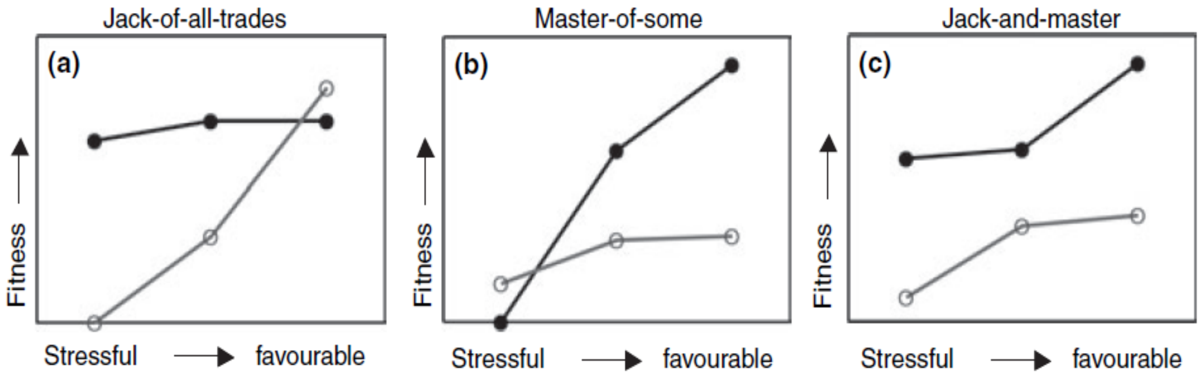

Fig. 5. Plasticité phénotypique et fitness attendue pour des espèces (ou génotypes) invasives (trait noir) et non-invasives (trait gris), d'après Richards et al. (2006). (a) *Jack-of-all-trades* : la fitness des invasives est plus robuste aux variations des conditions environnementales, conduisant potentiellement à une niche écologique plus large (homéostasie de la fitness) ; (b) *Master-of-some* : le gain de fitness lorsque les conditions sont favorables est plus important, ou vigoureux, chez les invasifs, permettant de plus fortes densités de populations ; (c) *Jack-and-master* : la fitness des invasifs suit une norme de réaction combinant à la fois la robustesse aux conditions environnementales et la vigueur.

Les réponses plastiques à un stress peuvent être de nature multiple, allant des ajustements physiologiques aux ajustements morphologiques et comportementaux (Stearns 1992 ; **ARTICLES II, V** et **VI**). Selon Willmer et al. (2000), ces ajustements peuvent être réversibles (*e.g.* acclimatation) ou non (plasticité développementale ; **ARTICLE II**) et décrire une gamme de variation continue ou discrète (*cf.* polyphénisme ; Moczek et al. 2002, de Jong et al. 2010). Willmer et al. (2000) définissent trois modes non exclusifs de réponse plastique aux conditions environnementales : (i) l'évitement, permettant de se soustraire au stress dans le temps ou dans l'espace (*e.g.* migration) ; (ii) la tolérance par arrêt du cycle biologique, impliquant la mise en état de dormance ou de quiescence (arrêt programmé ou non de l'activité, respectivement) ; (iii) la tolérance par conformité, impliquant l'ajustement de traits afin de maintenir l'homéostasie du milieu interne et donc l'activité malgré le stress. Lalouette (2009) indique que la tolérance et la régulation sont les bases du succès des espèces invasives (*cf.* également **ARTICLES V** et **VI**).

1.2.4.3. L'explosion à retardement des invasions

Williamson (1996) met en évidence le délai fréquemment observé entre la naturalisation d'une espèce et le début de son invasion proprement dite. Ce délai peut s'expliquer par les modifications de l'environnement ainsi que par les possibles changements évolutifs apparaissant progressivement après la naturalisation (Crooks 2005, Dietz & Edwards 2006 ; **partie 1.2.4.2.**), mais également par la non-linéarité de la pression d'invasion (Davis 2009). La pression d'invasion est une notion qui combine la pression de propagules à la probabilité d'invasion à partir de chaque propagule ; elle intègre donc implicitement l'invasibilité du milieu et les traits de vie individuels. Ce concept proposé par Davis (2009) traduit une augmentation non linéaire (ou phénomène *cliff*) du succès d'invasion si le nombre de propagules et leur probabilité de succès sont accrus (conjointement ou non). Cette relation non linéaire contribue à expliquer le retard temporel de l’expansion géographique d’une espèce introduite. Dans la nature, elle peut par exemple être associée à une population naturalisée encore trop petite pour produire le nombre de propagules suffisant à traverser la barrière d'invasibilité des environnements adjacents (Davis 2009).

D'autres phénomènes démographiques peuvent retarder l'explosion de la propagation d'une espèce, même en l'absence de phénomènes évolutifs (*i.e.*, si l'espèce est pré-adaptée ; Facon et al. 2006). En particulier, l'effet « Allee » décrit une situation dans laquelle une population de faible densité nuit à la fitness des individus et, donc, à sa propre croissance (Allee et al. 1949, Facon et al. 2006). Une faible densité de population rend en effet plus difficile la recherche de pollinisateurs ou de partenaires sexuels, diminuant de ce fait la fitness à l'échelle individuelle et réduisant par conséquent le taux de croissance à l'échelle populationnelle (Courchamp et al. 1999, Dérédec & Courchamp 2003, Tobin et al. 2007). Elam et al. (2007) ont ainsi illustré expérimentalement l'influence de la taille de population sur la fécondité chez une plante auto-incompatible (Fig. 6). Malgré son rôle dans de nombreux cas d'invasions (Genton 2005), l'effet « Allee » n'intervient pas de manière universelle dans les invasions biologiques. Fauvergue et al. (2007) ont ainsi suggéré son absence dans les cas d'invasions d'espèces ayant une grande capacité à trouver un partenaire sexuel et souffrant d'une intense compétition intraspécifique pour la ressource (densité-dépendance négative du taux de croissance), tels que les insectes parasitoïdes.

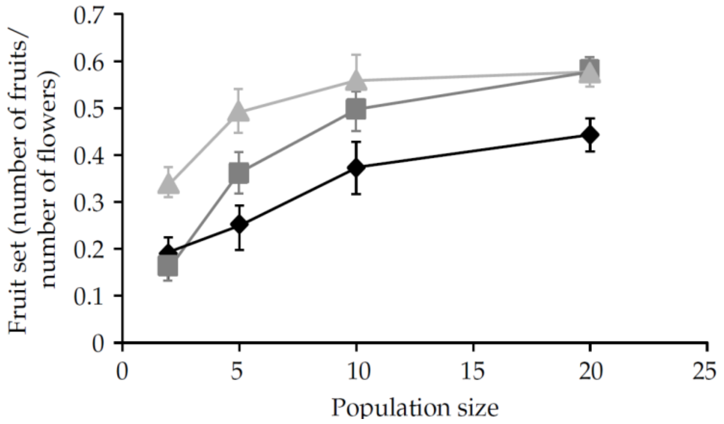

Fig. 6. Effets de la densité de population et du lien de parenté sur le nombre de fruits/nombre de fleurs (moyenne ± erreur standard), d'après Elam et al. (2007). Triangles : individus non apparentés ; carrés : individus ayant un parent commun ; losanges : individus ayant les mêmes parents.

Dans leur étude sur l'effet « Allee », Elam et al. (2007) ont également mis en évidence le rôle bénéfique de la variabilité génétique sur la fitness individuelle (Fig. 6). Ce résultat doit être mis en relation avec l'effet fondation et les goulots d'étranglement associés à l'étape d'introduction (Nei et al. 1975, Sax et al. 2007), démontrant le rôle facilitateur des introductions multiples qui augmentent le flux de gènes. D'un point de vue général, la combinaison de l'effet « Allee » et de l'effet fondation met en lumière l'influence de la pression de propagules déjà suggérée par Lockwood et al. (2005), ainsi que de la corrélation entre chances de succès et nombre d'individus introduits dans le milieu cible.

1.2.5. Tens rule, ou la probabilité de succès des espèces introduites

La prédiction des chances de succès des espèces introduites (autrement dit, leur probabilité de devenir invasives) constitue aujourd'hui l'un des enjeux majeurs de la biologie des invasions (Kolar & Lodge 2001, Hayes & Barry 2008). De nombreux paramètres ont ainsi été étudiés en tant que prédicteurs des invasions (Hayes & Barry 2008), depuis les traits de vie (Kolar & Lodge 2002 ; **partie 1.2.3.1.**) jusqu'à la phylogénie de la théorie de la naturalisation de Darwin (notamment la distance phylogénétique entre l'espèce introduite et les espèces qui constituent la communauté native ; Schaefer et al. 2011 ; **partie 1.2.3.2.**). La généralisation de ces relations apparaît toutefois fragile compte tenu de la complexité et de la diversité des contextes d'invasions biologiques. Il est ainsi vraisemblable que l'ensemble de ces facteurs interagissent perpétuellement les uns avec les autres, mais aussi avec des facteurs externes tels que la présence ou non d'ennemis naturels ou l'invasibilité du milieu, pour conditionner le succès des espèces invasives.

La *Tens rule* de Williamson (1996) correspond à une synthèse statistique générale des probabilités de succès des espèces à travers les phases clefs du processus d'invasion. Cet auteur estime qu'environ 10% des espèces introduites deviennent naturalisées et que, de ces espèces naturalisées, 10% parviennent à devenir invasives. Ces estimations approximatives peuvent grandement varier selon le taxon considéré, certaines études démontrant notamment des taux de naturalisation jusqu'à cinq fois plus importants (Jeschke & Strayer 2005, Hayes & Barry 2008). Elles sont par ailleurs largement tributaires des partis pris terminologiques assumés par les auteurs (***cf.*** **Avant-propos**), de grandes variations des mesures pouvant en effet résulter de définitions différentes des termes employés, comme le soulignent Jeschke & Strayer (2005). L'idée phare, uniformément généralisable aux invasions biologiques et qui doit selon nous être retenue de la règle de Williamson, est la notion de filtres successifs sélectionnant, de manière séquentielle au cours des étapes du processus d'invasion, une minorité d'organismes introduits.

1.2.6. Espèces invasives versus espèces natives, ou winners versus losers ?

Dans leur lettre publiée en 2011 dans *Trends in Ecology and Evolution* et intitulée *Why research on traits of invasive plants tells us very little,* Thompson & Davis mettent en avant l'importance primordiale des traits de vie des espèces dans la détermination du succès d'invasion. Derrière cet intitulé volontairement provocateur, ces auteurs insistent avant tout sur l'analogie classique entre les traits dits prédicteurs des invasions et les traits qui caractérisent les espèces natives rencontrant elles aussi un fort succès dans leur milieu. Selon Thompson & Davis (2011), bien que la perte des ennemis naturels ait un effet facilitateur, celle-ci est inévitablement temporaire dans la perspective de la sélection naturelle. Aussi, le succès des plantes dans les paysages actuels est avant tout gouverné par leurs histoires de vie, indépendamment de leur origine géographique, contribuant à accentuer la distinction entre phénotypes *winners* et *losers*. En effet, dans le contexte contemporain des perturbations massives et régulières de l'environnement (changements climatiques, eutrophisation, *etc.*), toute espèce peut être considérée comme vivant, à un moment ou à un autre, dans un environnement nouveau (Thompson & Davis 2011). Dans cet exemple, les espèces de plantes rencontrant un fort succès – ou *winners* – sont les espèces rudérales, parmi lesquelles se trouvent (et se trouveront toujours) à la fois des espèces natives introduites (Thompson & Davis 2011).

Classiquement associée aux variations du potentiel disperseur ou reproducteur, la différence entre *winners* et *losers* doit également être considérée en termes de plasticité phénotypique, qu'elle soit restreinte aux espèces invasives et natives ou non. En effet, le cadre théorique initialement proposé par Richards et al. (2006) comme hypothèse contribuant à expliquer le succès des espèces invasives (**partie 1.2.4.2.**) peut être plus généralement élargi au contraste existant entre organismes *winners* et *losers* mis en lumière par Thompson & Davis (2011), la plasticité phénotypique étant un élément essentiel de leurs stratégies de vie.

La vision de Thompson & Davis (2011) tend à remettre en question le sens écologique, dans les paysages anthropisés actuels, de la dichotomie entre espèces natives et introduites. Elle amène à la réflexion sur la politique de gestion des espèces dont la prolifération et la propagation ont des conséquences sanitaires, économiques ou écologiques néfastes, quelle que soit leur origine. Elle se limite toutefois aux paysages sous forte influence anthropique, restant difficilement applicable aux environnements isolés dont le régime de perturbations reste peu intense.

1.3. Contexte et objectifs de la thèse

1.3.1. Les espèces invasives : des modèles uniques sous-utilisés en biologie évolutive

Sax et al. (2007) rappellent dans leur synthèse que l'étude des espèces non-natives a contribué par le passé à la compréhension de nombreux concepts d'écologie évolutive, tels que l'adaptation, l'isolement reproducteur, la géographie de la spéciation ou le rôle de la dispersion dans la distribution des espèces. Selon ces auteurs, l'emploi des invasions biologiques pour traiter des processus fondamentaux de l'évolution et de l'écologie n'est réapparu que récemment (*cf.* Levin 2003, Huey et al. 2005, Sax et al. 2005, Cadotte et al. 2006, Roderick & Vernon 2009).

Les invasions biologiques peuvent être perçues comme des expérimentations à grandes échelles spatiale et temporelle, parfois répliquées, qui n'ont pas d'équivalent dans les manipulations de terrain ou de laboratoire conçues intentionnellement (Huey et al. 2005, Sax et al. 2007). L'évolution fait partie intégrante des invasions biologiques, avant même l'introduction dans le nouvel environnement, puisque des pressions sélectives opèrent déjà lors du transport des propagules et réduisent la gamme de phénotypes qui entrent en contact avec le nouvel environnement (Genton 2005, Novak 2007). De nouvelles pressions de sélection sont ensuite rencontrées à mesure que les espèces envahissent de nouveaux milieux (Huey et al. 2005, Novak 2007). Peuvent également intervenir des phénomènes épigénétiques (Schierenbeck & Aïnouche 2006) ou, chez les plantes, d'hybridation, ceux-ci ayant également un impact direct sur la reproduction des espèces natives (Ellstrand & Schierenbeck 2000). Ainsi, en l'absence de pré-adaptation ou de modification des caractéristiques du milieu cible, le succès d'une invasion repose sur les processus évolutifs intervenant après l'introduction (Facon et al. 2006). Par ailleurs, l'invasion peut à son tour modifier le fonctionnement des écosystèmes – si bien que de nombreux auteurs associent le terme « invasif » à l'effet négatif sur les communautés natives (Falk-Petersen et al. 2006) – et ainsi la sélection naturelle qui opère sur les espèces natives (Davis 2009 ; **ARTICLE III**). Les invasions biologiques représentent ainsi des systèmes privilégiés pour observer en temps réel l'évolution *in natura*, la prédire, étudier sa vitesse, ou encore traiter des problématiques de base de l'évolution et de la biogéographie (Huey et al. 2005, Novak 2007). La relation réciproque entre le milieu envahi et les espèces invasives, ainsi que la dynamique de l'invasion elle-même, sont perpétuellement influencées non seulement par le niveau de perturbation du milieu suite aux activités humaines, mais aussi par les réponses évolutives que développent les espèces invasives, leur compréhension est donc essentielle en écologie.

Cependant, Novak (2007) précise que la plupart des recherches sur les invasions biologiques se sont focalisées sur leur prédiction à travers les problématiques suivantes : quelles espèces deviendront invasives ? quels traits de vie contribuent au potentiel d'invasion ? quelles communautés sont invasibles ? quelles seront les conséquences écologiques des invasions ? Les réponses à ces questions sont souvent difficilement généralisables du fait de la nécessité de considérer conjointement l'espèce invasive et le milieu envahi (Mack et al. 2000, Kolar & Lodge 2001, Facon et al. 2006, Novak 2007 ; **partie 1.2.**). Peu d'auteurs se sont concentrés sur les paramètres évolutifs associés aux invasions et sur l'ajustement des traits fonctionnels en regard des contraintes du nouveau milieu (Lee 2002, Allendorf & Lundquist 2003, Lambrinos 2004, Facon et al. 2006, Novak 2007). **Ce manuscrit a pour objectif de contribuer à la meilleure compréhension de la relation étroite qui existe entre les traits fonctionnels des espèces invasives et les nouvelles contraintes environnementales dans les milieux envahis. L'adéquation de ces deux composantes, qui peut passer par la pré-adaptation, l'adaptation locale ou encore la plasticité phénotypique, est un paramètre clef du succès des espèces hors de leur aire d'origine.**

1.3.2. Les îles subantarctiques : des laboratoires naturels d'exception

Les îles océaniques sont des modèles naturels à l'origine de nettes avancées conceptuelles en écologie, notamment dans le secteur de la biologie évolutive (Simberloff 1974, Lomolino 2000). La pertinence des systèmes insulaires dans ces disciplines est notamment devenue paradigmatique suite aux travaux influents de MacArthur & Wilson (1963, 1967), en particulier l'*Equilibrium Theory of Island Biogeography*[1]. Cependant, l'emploi des écosystèmes insulaires comme situations simplifiées

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[1] Bien que certains auteurs la considèrent aujourd'hui trop simpliste dans sa forme originale, cette théorie a depuis été affinée et représente une rupture historique dans la discipline de la biogéographie (Lomolino 2000).

pour traiter des problématiques d'écologie évolutive est plus ancien encore : il peut déjà être attribué à Darwin pour ses observations sur l'archipel des Galapagos (Darwin 1859).

Par leur isolement géographique, leur fragilité et leur singularité (endémisme par exemple), les écosystèmes insulaires constituent des opportunités uniques pour une meilleure compréhension des invasions biologiques à différentes échelles spatiales et temporelles (Roderick & Vernon 2009, Simberloff 2009b). Roderick & Vernon (2009) indiquent que les invasions d'îles sont souvent documentées dès l'étape d'introduction, permettant ainsi l'étude des stages précoces de l'invasion, eux-mêmes représentant des références incontournables pour l'étude des changements écologiques et évolutifs mis en jeu au cours des invasions.

Parmi les écosystèmes insulaires, les îles subantarctiques ont été décrites par Bergstrom & Chown (1999) comme particulièrement sensibles et représentatives de la vulnérabilité aux changements globaux. Situées à proximité de la barrière climatique du front polaire (Fig. 5), ces terres sont fortement influencées par les masses d'eau froide environnantes et se caractérisent par un climat hyperocéanique froid et uniforme, avec peu de variations saisonnières (Chevrier 1996, Bergstrom & Chown 1999). En raison de leur isolement géographique extrême (Fig. 7), de leur sensibilité accrue aux introductions d'espèces et aux changements climatiques (Bergstrom & Chown 1999, Chown & Lee 2009), ainsi que de leur fréquentation humaine particulièrement faible et contrôlée, Bergstrom & Chown (1999) les considèrent comme des sentinelles permettant d'améliorer notre compréhension des changements globaux et d'anticiper leurs conséquences. Ces milieux se caractérisent par ailleurs par de faibles diversités spécifique et fonctionnelle des communautés d'invertébrés, facilitant la compréhension de l'influence des activités humaines et des changements environnementaux à long terme sur le fonctionnement des écosystèmes (Vernon et al. 1998).

Il est enfin important de remarquer que si les épisodes d'introductions multiples sont une règle plutôt qu'une exception selon Novak (2007), il est raisonnable de penser que cette règle ne s'applique que dans une moindre mesure dans les îles subantarctiques. En effet, bien qu'elles soient en augmentation, les pressions de propagules restent comparativement faibles dans la zone subantarctique du fait de la découverte récente des îles de cette région, de l'absence de populations permanentes et de l'absence de grandes voies d'échanges pour la plupart d'entre elles (Frenot et al. 2006 ; **ARTICLE I**). Décuplée lors de l'installation des bases scientifiques, l'activité anthropique reste néanmoins exceptionnellement faible et localisée en comparaison d'autres milieux insulaires (Frenot et al. 2001, Frenot et al. 2005, Chown et al. 2008). Certains cas d'introduction unique sont ainsi documentés dans les îles subantarctiques (Chevrier et al. 1997), simplifiant l'interprétation des processus éco-évolutifs qui peuvent intervenir au cours des invasions. Par ailleurs, le point d'entrée des propagules dans les îles subantarctiques est focalisé sur les lieux de débarquement des navires, soit essentiellement les bases scientifiques (Chown et al. 2005, Whinam et al. 2005, Convey & Lebouvier 2009, Renault 2011). Les bases correspondent donc à la fois aux habitats les plus fréquentés par l'Homme et les plus touchés par les espèces introduites, représentant un point de relais pour la dispersion des espèces introduites. En effet, les activités humaines depuis les bases ou leur périphérie jusqu'à des sites isolés sont un moteur essentiel de la dissémination secondaire de propagules dans les zones non anthropisées des îles subantarctiques.

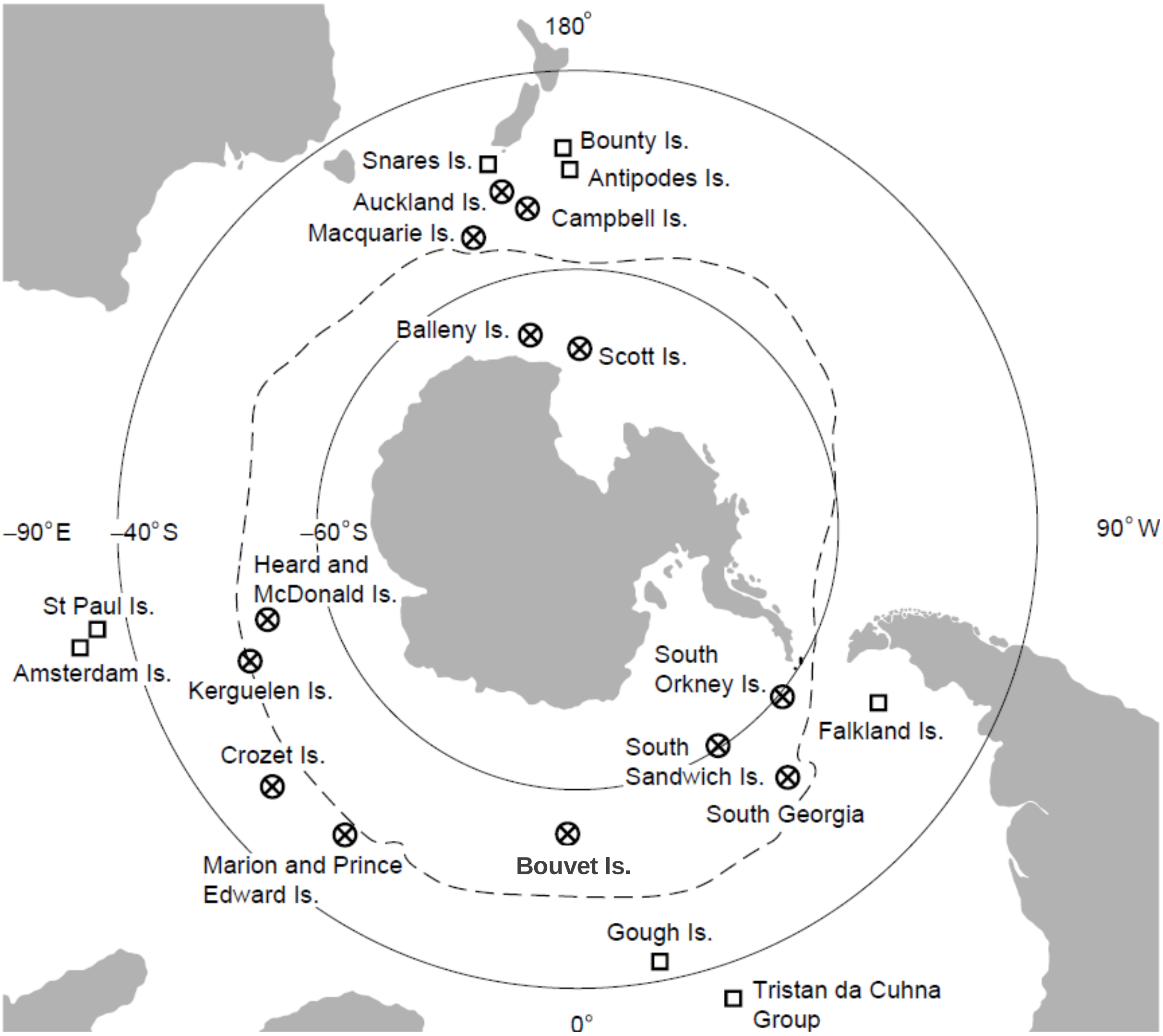

Fig. 7. Localisation géographique des îles subantarctiques et du front polaire (ligne pointillée), d'après Bergstrom & Chown (1999). Les îles marquées d'une croix sont celles qui se sont trouvées au sud du front polaire lors du dernier maximum glaciaire, il y a environ 16 000 ans.

1.3.2.1. Présentation du système d'étude : les insectes invasifs des Îles Kerguelen

Les Îles Kerguelen (48°27'–50°00' S, 68°27'–70°35' E) représentent le plus grand archipel subantarctique du globe avec une superficie de 7200 km² et plus de 300 îles et îlots, soit un total de 2800 km de côtes (Chown et al. 2008, Renault 2011 ; Fig. 8). Comme les autres îles subantarctiques, les îles Kerguelen présentent un isolement géographique extrême, un climat froid aux faibles variations saisonnières, des vents forts et récurrents ainsi que des précipitations élevées, sujettes à un intense gradient longitudinal (Chevrier 1996).

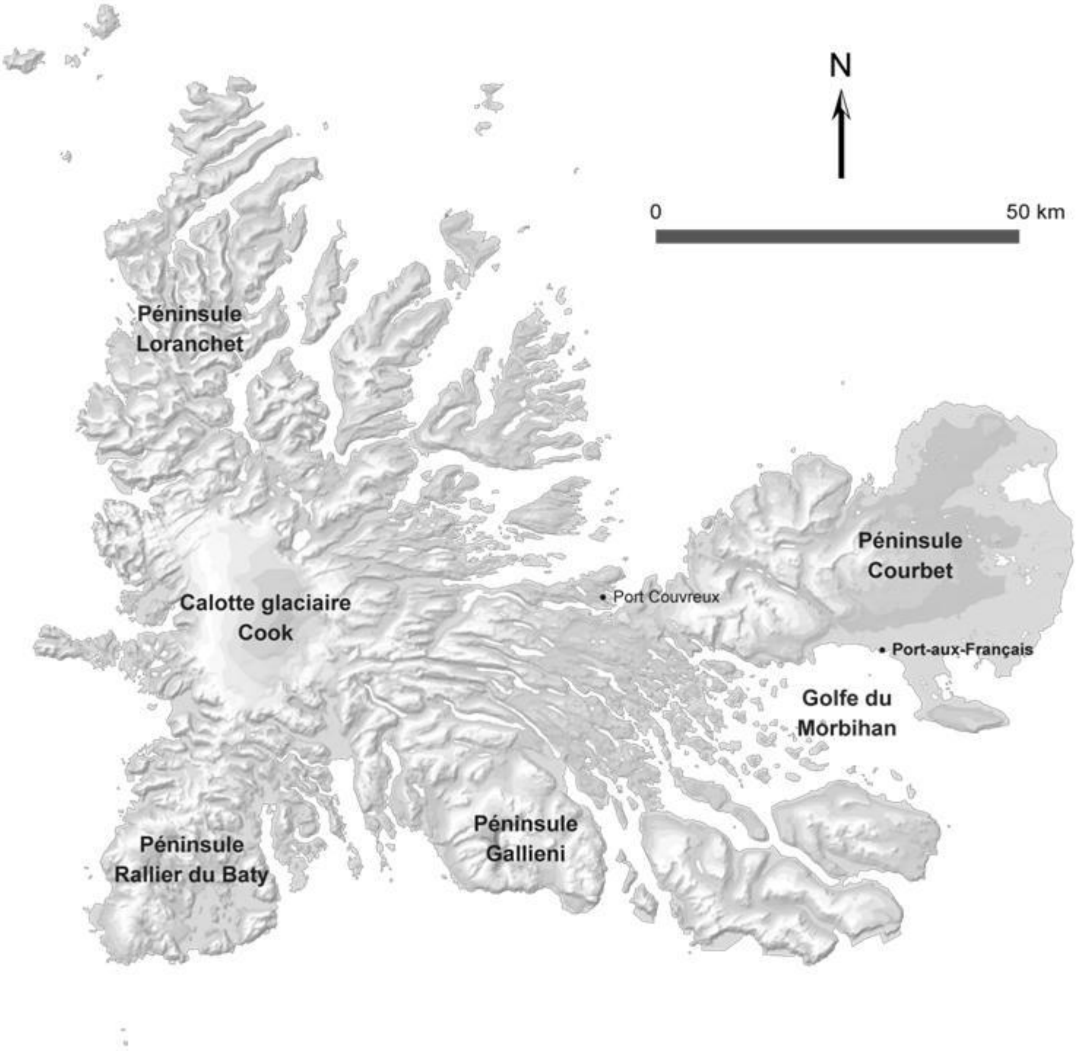

Fig. 8. Carte générale des principales îles et îlots de l'archipel de Kerguelen, modifiée et adaptée de la carte de Rémi Kaupp, CC-BY-SA, Wikimedia Commons.

Les écosystèmes terrestres de ces îles se caractérisent par des communautés d'invertébrés paucispécifiques (Chapuis et al. 2002), marquées par une dysharmonie des réseaux trophiques et une dominance de la guilde des décomposeurs (Crafford et al. 1986, Chown et al. 1998, Convey 2001). En effet, l'énergie et les nutriments qui entrent dans les systèmes terrestres subantarctiques proviennent majoritairement des apports massifs en matière organique (déjections, cadavres) issus des colonies de reproduction de vertébrés marins (Vernon et al. 1998). Outre la dominance des saprophages, le déséquilibre fonctionnel caractéristique des écosystèmes terrestres de ces îles se manifeste également par un faible niveau de phytophagie et la quasi-absence d'invertébrés prédateurs dans les communautés natives (Vernon et al. 1998). Seules trois espèces d'arthropodes prédatrices sont rencontrées dans les îles Kerguelen : le staphylin *Antarctophytosus atriceps* (Coleoptera, Staphylinidae) et les araignées *Neomaso antarcticus* (Araneae, Linyphiidae) et *Myro kerguelensis* (Araneae, Desidae). Cette dernière espèce est la plus abondante et est distribuée de manière continue dans de multiples habitats à travers l'archipel, tandis que les deux autres, de plus petite taille, ont une répartition discontinue et sont presque cryptiques.

En raison des activités anthropiques croissantes (plus grande base subantarctique, présence humaine en hiver) depuis leur découverte tardive en 1772 par le navigateur français Yves de Kerguelen de Trémarec (Delépine 1998, Frenot et al. 2006), les Îles Kerguelen sont devenues l'archipel subantarctique comportant le plus d'espèces naturalisées, en dépit d'une faible biodiversité native (Frenot et al. 2005). Frenot et al. (2005) comptent notamment 69 espèces de plantes introduites dans cet archipel. Bien que naturellement dépourvu de mammifères terrestres, l'archipel abrite également aujourd'hui de nombreux mammifères introduits tels que les rats, les mouflons, ainsi que de très fortes densités de lapins, de rennes, de souris et de chats (Convey & Lebouvier 2009). Par ailleurs, 26 espèces d'arthropodes ont été naturalisées dans les Îles Kerguelen, dont six sont devenues invasives et représentent des menaces sérieuses pour les écosystèmes natifs (Frenot et al. 2005). Pour répondre aux problématiques de cette thèse, deux exemples d'insectes invasifs des Îles Kerguelen ont été sélectionnés en raison de leurs invasions documentées avec précision et de leurs caractéristiques biologiques particulièrement intéressantes dans le contexte subantarctique : le carabique *Merizodus soledadinus* Guérin-Méneville 1832 (Coleoptera, Carabidae) et la mouche bleue *Calliphora vicina* Robineau-Desvoidy 1830 (Diptera, Calliphoridae).

Ces deux cas d'invasions biologiques constituent sous plusieurs aspects des opportunités de recherche singulièrement complémentaires. Tout d'abord, la répartition de *M. soledadinus* et *C. vicina* fait l'objet d'un suivi à long terme depuis leur introduction dans l'archipel. De plus, les stratégies d'histoires de vie distinctes de ces deux espèces peuvent intervenir de manière différentielle sur leur impact et les raisons de leur succès invasif sous les latitudes subantarctiques. Enfin, le climat caractéristique, sa grande sensibilité aux changements climatiques (Bergstrom & Chown 1999 ; **ARTICLE I**) ainsi que la faible diversité des écosystèmes envahis simplifient la compréhension des causes et des conséquences de ces invasions.

1.3.2.1.1. Le carabique *Merizodus soledadinus* Guérin-Méneville 1832

Merizodus soledadinus est un carabique prédateur (Fig. 9) connu de Patagonie et des Îles Malouines (Jeannel 1940, Johns 1974, Chevrier 1996, Convey et al. 2010). Cet insecte a été observé dans les Îles Kerguelen pour la première fois en 1939 par René Jeannel (Jeannel 1940). Il a été introduit accidentellement en 1913 lors de l'importation de moutons et de fourrage en provenance des Îles Malouines pour la mise en place d'un élevage sur le site de Port Couvreux, à une époque où la valorisation des Îles Kerguelen était un enjeu essentiel dans la souveraineté française de ces terres australes (Bossière 1928, Jeannel 1940, Jeannel 1964). Cet épisode d'introduction unique, dans un seul site, représente un exemple rare et simplifié d'invasion biologique. Renault (2011) indique que la dispersion de cette espèce repose essentiellement sur la locomotion à micro-échelle mais peut également intervenir de manière passive, à travers des bras de mer, des cours d'eau, ou le transport de matière organique par les oiseaux natifs. La répartition de cette espèce a explosé à la fin des années 1990 (Lalouette et al. 2011 ; **ARTICLE I**), notamment depuis que l'espèce a envahi la base scientifique de Port-aux-Français, bénéficiant alors probablement des activités humaines comme nouveaux vecteurs de colonisation. Cette espèce a également été accidentellement introduite (probablement par les activités baleinières) et déclarée invasive en Géorgie du Sud (première observation en 1963 ; Darlington 1970). Cette autre île subantarctique est caractérisée par un climat plus rigoureux ainsi qu'une compétition interspécifique plus intense, notamment en raison de l'invasion d'un autre carabique prédateur, *Trechisibus antarcticus* (Coleoptera, Carabidae). Ces plus fortes contraintes limitent probablement l'invasion de *M. soledadinus*, beaucoup moins étendue que dans les Îles Kerguelen (Ernsting 1993, Todd 1997, Brandjes et al. 1999, Convey et al. 2010).

Fig. 9. Individus adultes de *Merizodus soledadinus* s'attaquant à des larves d'*Anatalanta aptera* (Diptera, Sphaeroceridae), une mouche native des Îles Kerguelen.

La stratégie de vie de *M. soledadinus* converge en de nombreux points vers celles des insectes natifs des îles Kerguelen. En effet, bien qu'il exploite une niche trophique sous-représentée, il colonise les nouveaux sites essentiellement de proche en proche en raison de son inaptitude au vol (ou bien par flottaison sur des cours d'eau ou des bras de mer ; Renault 2011), présente un développement continu tout au long de l'année avec une stratégie de type *K* et est originaire de la zone tempérée froide australe (Ernsting 1993, Chevrier 1996). Chevrier (1996) suggère que la naturalisation de cette espèce a été favorisée à la fois par la similarité des conditions climatiques entre ses habitats d'origine et d'introduction et par le nombre réduit de compétiteurs trophiques dans les Îles Kerguelen.

Le succès de cette espèce dans les Îles Kerguelen repose probablement en partie sur sa pré-adaptation aux conditions subantarctiques. L'étude de la tolérance thermique de *M. soledadinus* réalisée par Lalouette et al. (2011) appuie cette hypothèse, puisque les préférences thermiques de cette espèce correspondent étroitement aux conditions rencontrées localement dans les Îles Kerguelen. Ce carabique présente en effet un niveau de tolérance au froid compatible avec les conditions climatiques froides et tamponnées de la zone envahie (Lalouette et al. 2011). Ces auteurs ont néanmoins déterminé chez cette espèce que les températures critiques maximales sont bien plus élevées que les températures rencontrées lors des mois les plus chauds de l'été dans les Îles Kerguelen, démontrant que, bien que limitée, la plasticité thermique de cette espèce lui offre une marge confortable pour supporter les changements climatiques, très prononcés sous les latitudes subantarctiques (Bergstrom & Chown 1999).

1.3.2.1.2. La mouche bleue *Calliphora vicina* Robineau-Desvoidy 1830

Calliphora vicina est une espèce nécrophage cosmopolite, observée essentiellement dans les régions tempérées, mais également présente de la Patagonie jusqu'aux régions arctiques (Nuorteva 1967, Vinogradova 1986, Shewell 1987, Chevrier 1996, Schnack et al. 1998, Schnack & Mariluis 2004, Mariluis et al. 2008). Accidentellement introduite par voie maritime, cette espèce a été observée pour la première fois en 1978 à la base scientifique de Port-aux-Français par François-Xavier de Blignières (Chevrier 1996). En 1979 et 1980, des mouches bleues ont été observées dans divers bâtiments et dans le voisinage immédiat de la base scientifique (L. Davies, données non publiées). La localisation de la population d'origine est inconnue, mais il est vraisemblable que les individus introduits aient été transportés depuis Le Port (Île de La Réunion), le Canal de Suez, ou encore Brest ou Marseille (France métropolitaine), où se trouvent les ports d'attache principaux des bateaux scientifiques, militaires et de pêche amenés à mouiller dans le Golfe du Morbihan, à Port-aux-Français.

Cette mouche occupe une fonction trophique déjà largement représentée dans les communautés natives, la saprophagie, mais, à la différence de ses compétiteurs subantarctiques, *C. vicina* se déplace par le vol (Fig. 10) et possède de grandes capacités de dispersion (MacLeod & Donnelly 1958, Chevrier 1996). Elle présente également un cycle de développement saisonnier (diapause hivernale) avec une stratégie de type *r* et, bien que présente dans des régions froides, elle n'en est pas spécialiste (Chevrier 1996). Les caractéristiques ubiquistes de cette espèce ainsi que l'abondance des ressources trophiques dans les Îles Kerguelen ont fortement contribué à la naturalisation de cette espèce selon Chevrier (1996). Cependant, à l'inverse de celui de *M. soledadinus*, le succès invasif de *C. vicina* repose vraisemblablement en partie sur les changements écologiques (*cf.* Facon et al. 2006) intervenus dans les Îles Kerguelen à la fin des années 1970, notamment le réchauffement climatique (**ARTICLE I**), plutôt que sur la pré-adaptation. Cette espèce bénéficie de l'augmentation des températures qui lui permet de compléter son cycle de développement (*cf.* limites thermiques de développement dans Vinogradova 1991, Davies & Ratcliffe 1994 ; **ARTICLE II**), ainsi que de la vulnérabilité de ses compétiteurs natifs face aux changements climatiques (**ARTICLE I**).

Fig. 10. Femelle adulte de la mouche bleue *Calliphora vicina* et, en bas, femelle adulte de l'espèce native principale compétitrice, la mouche aptère *Anatalanta aptera.*

1.3.2.2. Vulnérabilité des Îles Kerguelen aux changements globaux (**ARTICLE I**)

L'article suivant justifie l'emploi des îles subantarctiques comme outils méthodologiques à part entière pour l'étude des invasions biologiques. À travers plusieurs exemples d'invasions dans les Îles Kerguelen, il expose les raisons de la vulnérabilité de ces milieux, les paramètres ayant contribué au succès des espèces introduites et décrit certains des changements écologiques qu'ont occasionné ces invasions récentes. Cet article présente notamment le rôle fondamental des changements climatiques dans la facilitation des différentes phases du processus d'invasion. Combinant revue de littérature et données nouvelles, ce travail met en évidence la singularité des Îles Kerguelen comme modèles d'étude des changements globaux. Il présente en effet les multiples particularités qui en font, selon certains auteurs, des laboratoires naturels dont les enseignements peuvent être généralisés à des systèmes d'études plus complexes (Bergstrom & Chown 1999).

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ORIGINAL PAPER

The significance of the sub-Antarctic Kerguelen Islands for the assessment of the vulnerability of native communities to climate change, alien insect invasions and plant viruses

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Abstract The suite of environments and anthropogenic modifications of sub-Antarctic islands provide key opportunities to improve our understanding of the potential consequences of climate change and biological species invasions on terrestrial ecosystems. The profound impact of human introduced invasive species on indigenous biota, and the facilitation of establishment as a result of changing thermal conditions, has been well documented on the French sub-Antarctic Kerguelen Islands (South Indian Ocean). The present study provides an overview of the vulnerability of sub-Antarctic terrestrial communities with respect to two interacting factors, namely climate change and alien insects. We present datasets assimilated by our teams on the Kerguelen Islands since 1974, coupled with a review of the literature, to evaluate the mechanism and impact of biological invasions in this region. First, we consider recent climatic trends of the Antarctic region, and its potential influence on the establishment, distribution and abundance of alien insects, using as examples one fly and one beetle species. Second, we consider to what extent limited gene pools may restrict alien species' colonisations. Finally, we consider the vulnerability of native communities to aliens using the examples of one beetle, one fly, and five aphid species taking into consideration their additional impact as plant virus vectors. We conclude that the evidence assimilated from the sub-Antarctic islands can be applied to more complex temperate continental systems as well as further developing international guidelines to minimise the impact of alien species.

Keywords Colonisation · Endemic species · Environmental variables · Human impact · Introduced species · Island communities · Species richness

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Introduction

Since the twentieth century, human-mediated movement of species to regions outside their natural geographical distribution has increased significantly (Hänfling and Kollmann 2002; Murphy and Cheesman 2006). The introductions of non-native species have resulted in substantial ecological damage through their increasingly greater alteration of terrestrial, marine and freshwater communities, and thus are becoming a worldwide ecological and conservation concern (Kolar and Lodge 2001; Mooney and Cleland 2001; Richardson and Pysek 2008; Simberloff 2003; Streftaris and Zenetos 2006). Anthropogenic disturbances frequently confer competitive advantages to alien species (Byers 2002), often leading to the extinction of native flora and fauna (for examples see Clavero and García-Berthou 2005; Gurevitch and Padilla 2004). However, not all established aliens have negative effects on local/regional species diversity, or the composition and functioning of communities. The impact of aliens depends on the context of each biological invasion, i.e. the invasiveness of the alien and the invasibility of the habitat (Gurevitch and Padilla 2004; Hoffmeister et al. 2005; Kenis et al. 2009; Levine et al. 2003; Strayer et al. 2006; Williamson 1996).

The ecosystems of oceanic islands are often described as more invasible and vulnerable habitats to biological invasions than those of continental ecosystems (Herben 2005), partly because many islands are still ecologically non-saturated (Williamson 1999). Paradoxically, the vulnerability of oceanic islands is often associated with an increased level of biodiversity, resulting from the progressive dominance of alien species (Borges et al. 2006). Several complementary causes have been proposed to explain the high susceptibility of oceanic islands to biological invasions (Blondel 1995; Chown and Gaston 2000; Connor and McCoy 1979; Roderick and Vernon 2009; Simberloff and Martin 1991). Prominent examples include (1) the naivety of native species to a wide range of aliens (Gillespie 1999; Gillespie and Roderick 2002), and (2) the establishment and spread of human-introduced species without being controlled by natural enemies (see Colautti et al. 2004; Liu and Stiling 2006 for more details on the Enemy Release Hypothesis). In addition, areas with richest amounts of endemic species should exhibit higher levels of alien species (Borges et al. 2006). Interestingly, at the Southern Ocean Islands, which are considered to be some of the most sensitive ecosystems to biological invasions (Chown and Lee 2009), a similar conclusion was reported.

The sub-Antarctic province form a loose ring within the latitudinal belt 45–54°S (Whinam et al. 2005), where the Antarctic Polar Frontal Zone (APFZ), in the South of the area, constitutes a major oceanic and climatic boundary. These are amongst the most isolated islands from any continental landmass and contain a number of the limited terrestrial habitats present at these latitudes (Bergstrom and Chown 1999, Chown et al. 2008a), and as such have been considered as natural laboratories (Carlquist 1974). There are very few species of fauna and flora, with many familiar taxonomic and functional groups of other latitudes being absent (Bokhorst et al. 2007; French and Smith 1985; Gillespie and Clague 2009). As an example, to date, 210 terrestrial insect species, including more than 90 collembolan species (i.e. springtails), have been described inhabiting the sub-Antarctic islands (Huiskes et al. 2006). These impoverished terrestrial ecosystems are thus simple, with highly reduced or absent functional redundancy. Food webs are poorly developed, with a relatively high number of decomposers and very few herbivores or predators (Chown et al. 1998; Convey 2001; Crafford et al. 1986; Gressitt 1970; 1971; Vernon and Voisin 1990; Vernon et al. 1998; Watson 1967). These ecosystems rely heavily on the surrounding oceanic waters, and are enriched by nutrient inputs of marine origin, due to the presence of numerous seabirds and seals coming ashore to moult and/or reproduce (Smith 2008). Despite their remoteness, these islands have been subject to colonisation by high numbers of alien species (Chown et al. 2005; Frenot et al. 2001; Gaston et al. 2003). The consequences of species introductions, the extinction of native species and/or their complex co-existence may lead to biological homogenisation or differentiation (Olden and Poff 2003; Shaw et al. 2010). Recently, the taxonomic homogenisation of native and alien insect assemblages has been convincingly demonstrated on sub-Antarctic islands by Shaw et al. (2010), who stressed that this kind of biotic homogenisation "may lead, *inter alia*, to food web simplification, declining resistance to environmental change and susceptibility to further invasions (Olden et al. 2004)".

The physical and climatic remoteness of the French sub-Antarctic Kerguelen Islands (48°30′–50°S, 68°27′–70°35′E), together with their comparatively low human visitation rates, have hindered biological invasions until the recent establishment of a permanent station in 1950 and the subsequent increase in visiting ships and visitors landing (Chown et al. 1998, Frenot et al. 2005). Aliens are mainly, or even exclusively, introduced to the Kerguelen Islands by ship (there is no airport), with most introductions occurring at the research station (Port-aux-Français). In addition, the Kerguelen Islands are not situated along major commerce routes, are inhabited by a limited number of non-permanent people each year (at most 100), do not have any industry and have very limited means of terrestrial transportation that would facilitate quick and regular exchanges between geographically distant points for an alien species. Overall, the Kerguelen Islands are characterized by low species diversity, supporting just 23 native insect species (Schermann-Legionnet et al. 2007) and 22 native flowering plant species (Van der Putten et al. 2010). It is therefore reasonably straightforward to detect the arrival and establishment of alien insect species.

In this review we argue that sub-Antarctic islands provide an unrivalled opportunity to address the topic of biological invasion, using evidence from the research already conducted on the Kerguelen Islands. Arthropods, and in particular insects, represent more than 63% of alien animal species on the Kerguelen Islands (Frenot et al. 2005), hence in this review we focus on examples of three insect taxa; one ground beetle species *Merizodus soledadinus* (Coleoptera: Carabidae), one fly species *Calliphora vicina* (Diptera: Calliphoridae) and five aphid species (Homoptera: Aphididae) (Table 1). These aliens have distinct nutritional needs, life cycles, and dispersal capabilities, and as such provide an integrative view of their potential impact on native communities. In addition, these species are nowadays widely distributed across the Kerguelen Islands and are the most likely to have a significant impact on native communities (for more details see Frenot et al. 2005, Schermann-Legionnet et al. 2007). Through the collation of the original data obtained from our long-term field survey of the biota, together with data from the literature, we discuss (1) the evolution of the distribution and abundance of these alien insects in light of climate change, and (2) the vulnerability of native communities to climate change and alien insects on the Kerguelen Islands. As available data about introduced species mainly deal with visible ones (plants, invertebrates, vertebrates), we also discuss the possible impact of invisible aliens, and focus on plant viruses that can be vectored by insects.

To what extent does climate change favour the establishment and dispersal of alien insects?

Thermal and precipitation trends on the Kerguelen Islands over the last 60 years

The temperature changes documented in the maritime Antarctic and the sub-Antarctic are amongst the most rapid worldwide (Bergstrom et al. 2006; Smith 2002). During the last six decades on the Kerguelen Islands, a 1.9°C difference in mean air temperature has been recorded between the coolest year (1964, 3.5°C) and the warmest year (1982, 5.4°C) (Fig. 1a). Mean annual temperatures were cooler and more variable over the period 1951–1975 (4.30°C; Standard Deviation: 0.44°C) compared to 1976–2008 (4.84°C; SD: 0.27°C) (Fig. 1a).

In parallel, highly significant reductions in total annual precipitation levels have been observed on the Kerguelen Islands since the early 1990s (Fig. 1b).

Table 1 Detailing the host plants and landscape distribution of the five aphid species introduced to the Kerguelen Islands

	Host plants		Landscape distribution	
	Native	Non-native	Wild	Greenhouse*
Aulacorthum solani	0	3	First occurrence in 2008	Until 2009
Myzus ascalonicus	5	21	Common	Until 2009
Myzus ornatus	0	2	Expanding	Absent
Myzus persicae	0	5	Suspected	Until 2009
Rhopalosiphum padi	3	14	Apparently regressing	Absent

* Cultivations stopped in 2009

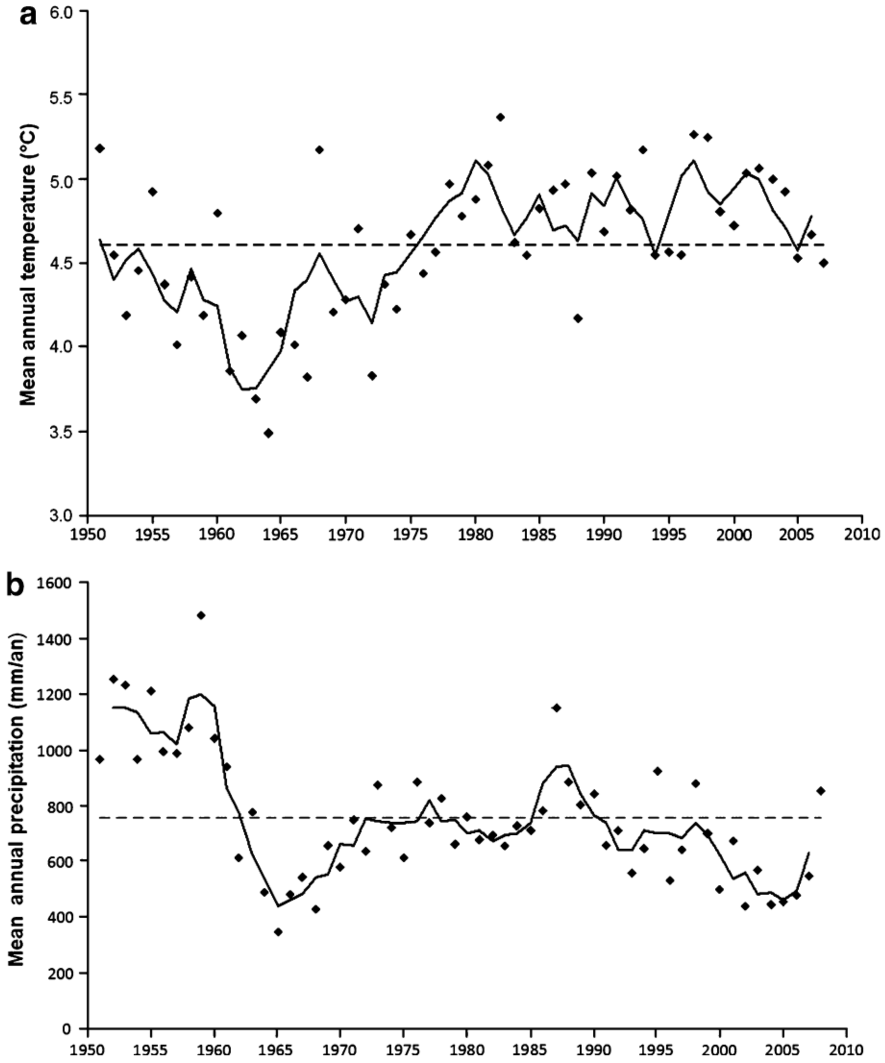

Fig. 1 Annual mean air temperature (°C, **a**) and annual precipitation (mm y^{-1}, **b**) at the Kerguelen Islands (Port aux Français) over the period 1951–2008. The *broken lines* indicate the average annual temperature (**a**) and precipitations (**b**) for the period 1951–2008; the *solid lines* represent the 3-year running mean. *Source:* Météo France data

Chown and Smith (1993), and more recently Smith (2002), also found that precipitation levels were markedly reduced during the 1960s and 1970s on Marion Island, with the 1990s being the driest years on record. Because there are many soil arthropods on the sub-Antarctic islands, we compared the thermal evolution of air and soil temperature over a period of years (Fig. 2). We found that even at a depth of 20 cm below ground level, both air and soil temperature remained very similar. In addition, an increase in usually constant cool thermal conditions, in parallel to a reduction in precipitation levels, has resulted in a significant reduction in the number of annual freezing days on the Kerguelen Islands (Fig. 3).

The example of the flight-capable fly Calliphora vicina and the flightless ground beetle Merizodus soledadinus

The flight-capable blue blowfly, *C. vicina*, is widespread in temperate regions of the globe, but also occurs in regions with more severe climatic conditions. *Calliphora vicina* has been recorded in both the Arctic and Patagonia (Mariluis et al. 2008; Nuorteva 1967; Schnack et al. 1998; Schnack and Mariluis 2004; Shewell 1987). This necrophagous fly was accidentally introduced to the Kerguelen Islands and was first observed in 1978 in Port-aux-Français (Chevrier 1996; Chevrier et al. 1997). Since then, *C. vicina* has spread across the eastern part of the

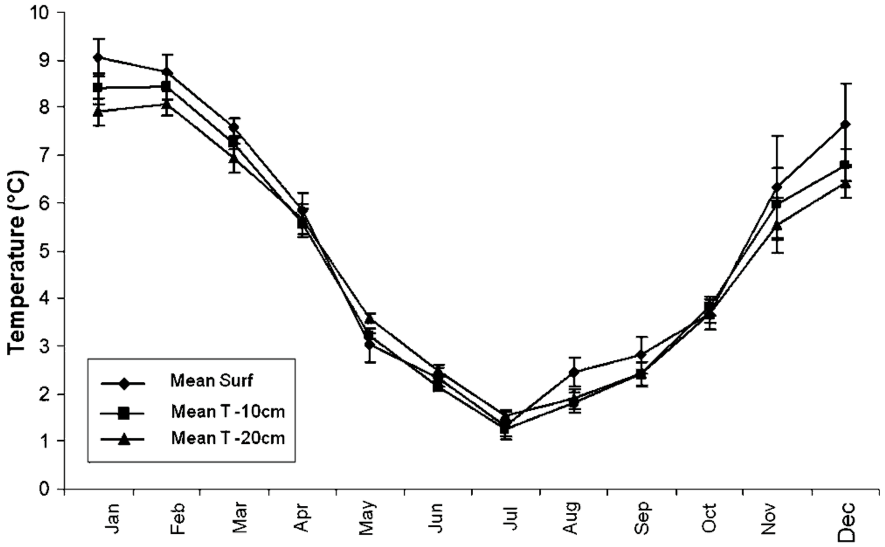

Fig. 2 Time series of mean monthly temperatures (±SEM) at the Kerguelen Islands (Port aux Français) from 2000 to 2005. Temperatures (°C) were recorded at the ground level, 10 and 20 cm below the ground level with a Delta T data logger (Delta T devices, Cambridge, UK)

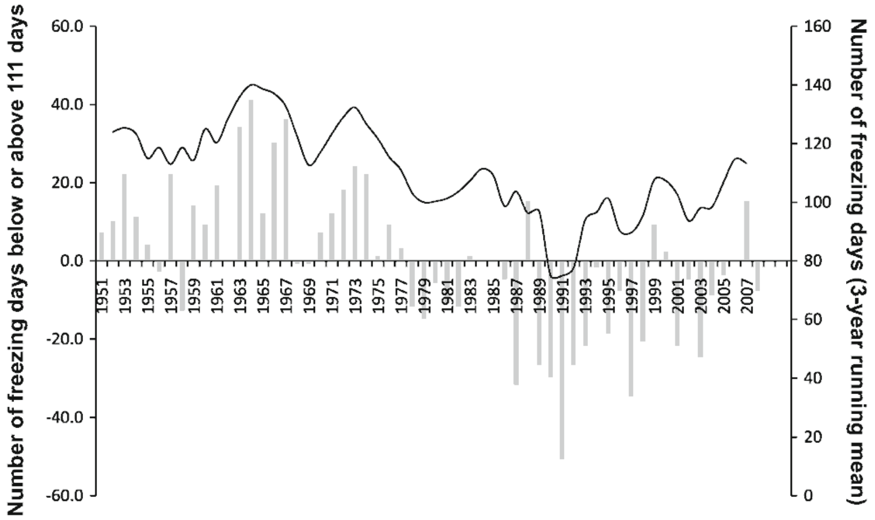

Fig. 3 Number of annual freezing days below or above the average for the period 1951–2008 (111 days). The *solid line* represents the 3-year running mean. *Source:* Météo France data

archipelago (Fig. 4), including many islands within the Golfe du Morbihan. Flying individuals were first observed in the western part of the archipelago (Baie Caillé) during the austral summer 2010–2011, thus extending the known range of *C. vicina* at the Kerguelen Islands further to the North-West between the austral summer 2009–2010 and 2010–2011 (Fig. 4). The flying activity of *C. vicina* has been directly correlated with daily climatic conditions (Chevrier et al. 1997), as well as solar radiation and relative humidity parameters (Vogt 1988). Indeed, the window of opportunity for flying activity may be linked to a reduction in the level of annual rainfall, whereby flight ability significantly increases during drier years that remained thermally similar.

During the 1970s, a period of rising air temperature had a major influence on the establishment of *C. vicina* populations on the Kerguelen Islands, facilitating the successful completion of this species' life cycle. Individuals of *C. vicina* originating from the Kerguelen Islands and reared at a range of controlled temperatures from +8 to +20 °C were characterised by (1) an unaltered life cycle (except for requiring a longer period for development at cooler temperatures), (2) no variation in life cycle compared to individuals from temperate areas (metropolitan France) when reared under the same conditions and (3) no impact of developmental temperature on the body mass of the third larval instars at their exodus, when the population density remained constant. These results, based on *C. vicina* strains acquired from the Kerguelen Islands and metropolitan France, were consistent with the available literature (Davies and Ratcliffe 1994). While body mass often varies along climatic gradients in a large number of insect orders (see the temperature-size rule, Atkinson 1994; Kingsolver and Huey 2008), in *C. vicina* body mass seems to exhibit low levels of variation in response to developmental temperatures. Hence, global warming serves to both increase flying activity and magnify the developmental processes of this invasive species. This would facilitate a higher number of generation cycles each year. If climate change has already facilitated the establishment of *C. vicina* on the Kerguelen Islands, any further rise in air temperature would continue to favour the population dynamics of this species, possibly leading to a tremendous increase in population density on these islands.

Native populations of the ground beetle, *Merizodus soledadinus*, are only known from the southern cold temperate zone, in Patagonia and on the Falkland Islands (Darlington 1970; Johns 1974). This species was first observed on the Kerguelen Islands in February 1939 (Jeannel 1940), but was probably introduced at one site in 1913 (Port Couvreux, see Fig. 4) when sheep and fodder were imported from the Falkland Islands. This polyphagous flightless beetle initially remained restricted to the vicinity of its initial introduction site, with a gradual colonisation of coastal habitats along the northeast coast of the Kerguelen Islands (Péninsule Courbet). In the 1990s,

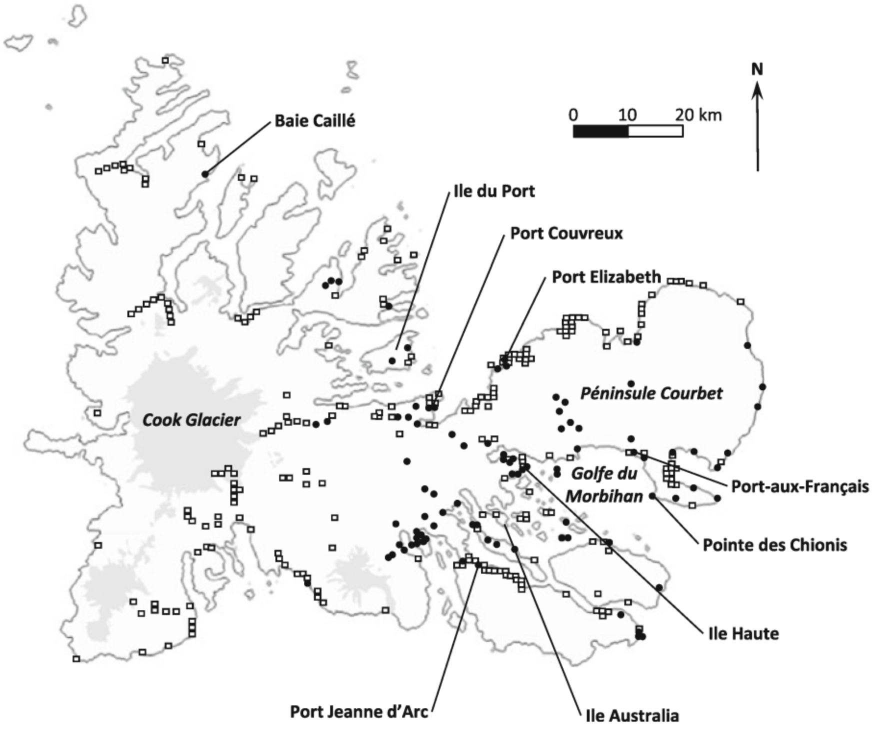

Fig. 4 Current geographic distribution of *Calliphora vicina* at the Kerguelen Islands. *Open squares*: locations where individuals were searched but not observed; *Black dots*: locations where individuals were observed (no attractive traps were used, to ensure that observed individuals were naturally active in the sites). The main sites of Kerguelen Islands discussed in this review are presented in this figure

M. soledadinus started to colonise new locations (i.e. Port Jeanne d'Arc, Ile Haute) that were remote from the original point of introduction (Chevrier et al. 1997). Since then, there has been a significant increase in the speed of the colonisation process. Numerous sites far from Port Couvreux have been subject to colonisation, including Port-aux-Français (the research station), the seashore and several islands in the Golfe du Morbihan. Of particular note, the abundance of *M. soledadinus* had significantly increased between 1995 and 2005 in several habitats, from tens to several hundred individuals per 10 min search period per person (Laparie et al. 2010).

Merizodus soledadinus has also been introduced to South Georgia, another sub-Antarctic island, where it was first observed in 1963 (Darlington 1970). This species has only colonised a limited area in South Georgia (Ernsting 1993), in comparison to its wide distribution on the Kerguelen Islands. Furthermore, on Ile Haute (Kerguelen Islands), where *M. soledadinus* was first recorded in 1992, the geographical spread of this flightless six-millimetre long insect was estimated to be 3 km y^{-1} (Chevrier 1996), versus 0.1 km y^{-1} on South Georgia (Brandjes et al. 1999). Moreover, lower abundances of *M. soledadinus* were registered on South Georgia, ranging from 29 to 156 collected per hour per person (Brandjes et al. 1999). These differences may be attributed in part to the distinct climates of these two islands. For example, the mean annual temperature recorded at King Edward Point (South Georgia) in 1951–1980 was about 2°C, with mean monthly temperatures varying from +5.6°C in February to −1.5°C in July (Headland 1984). During the same period, the mean annual temperature at the Kerguelen Islands was about +4.4°C, with mean monthly temperatures varying from +7.4 (SD: 0.9°C) in February to +1.9 (SD: 0.8°C) in July.

Many invertebrates that have been introduced to this extreme environment were thus exposed to chronically low temperatures, close to the limits of their operational thresholds. Thus, even a small thermal increase (i.e. in the range of 1°C) may have important biological consequences, potentially driving the success or failure of the establishment of alien species in sub-Antarctic regions. Hence any rise in temperature is likely to enhance the ability of human-assisted aliens to become established, or to switch from a status of 'persistent' to one of 'invasive' (Bergstrom and Chown 1999; Frenot et al. 2005, 2006). Therefore, for both flora and fauna, a slight thermal increase is likely to have a far greater impact in sub-Antarctic regions than in warmer regions (Convey 2001; Convey et al. 2002). Conversely, invertebrates that are endemic to sub-Antarctic regions, and hence highly adapted to a cool climate, are particularly vulnerable to slight increases in thermal conditions (Chwedorzewska 2009; Convey 1996a, b, 2001). This in turn magnifies any competitive advantage held by alien species', even if also originating from a cool region, such as *M. soledadinus*.

To what extent do molecular studies indicate the genetic capacity of alien species for colonisation?

Several factors make the sub-Antarctic islands viable candidates to develop molecular ecology studies (Stevens and Hogg 2006, Chown et al. 2008b). First, the remoteness of the islands limits the influx of populations from the continents. Second, introduction events of alien species have been well documented (for review see Convey and Lebouvier 2009; Frenot et al. 2005). Third, the biota on these islands has been subject to long-term monitoring programmes (Chown et al. 1998, 2009; Cooper et al. 2009; Convey and Lebouvier 2009; Frenot et al. 2001). Sub-Antarctic Islands could therefore potentially facilitate valuable investigations with respect to the patterns of genetic diversity, colonisation pathways and possible post-bottleneck genetic diversification.

Aphids present interesting models for molecular studies as they are strictly parthenogenic on the Kerguelen Islands (Delmotte et al. 2003; Hullé et al. 2003a), as has also been documented on Crozet (Hullé et al. 2003b) and Macquarie Islands (Greenslade 2006). A reduced allelic diversity at the microsatellite loci was found in asexual versus sexual lineages (Delmotte et al. 2001, 2003). Among the five introduced aphid species to the Kerguelen Islands, *Myzus ascalonicus* and *Rhopalosiphum padi* produce a larger proportion of winged individuals than the other three species. This allows larger dispersal capabilities, while also causing an increase in the fragmentation of these populations. On the Kerguelen Islands, genetic studies were conducted to determine the colonisation pathways of *R. padi*, in association with how different dispersal processes and parthenogenesis may affect genetic variability and composition (Simon et al. 2001). Several populations of *R. padi*, separated by up to several tens of kilometres, were sampled. The assimilated data revealed that only two genotypes exist (Delmotte et al. 2001). The first genotype was found at Port aux Français, Ile Australia, Pointe des Chionis and Port Couvreux (See Fig. 4 for the localisation of these sites). This genotype was also found at Possession Island in the Crozet Archipelago. The second genotype was only found at Pointe des Chionis, the habitat where *R. padi* was most abundant in the past. The second genotype differs from the first by just one of the eight microsatellite markers that were tested (Delmotte et al. 2003). This limited genetic diversity and the presence of the first genotype at the Kerguelen and Crozet Islands, both visited by the same supply ship, suggest that populations of *R. padi* at the Kerguelen Islands have likely radiated from one initial introduction site of a few individuals at Port aux Français. Interestingly, in the last 3 years no records of *R. padi* have been made at Pointe des Chionis, suggesting a strong bottleneck effect that may have resulted in the extinction of this population.

Microsatellite markers have been recently developed for the introduced ground beetle, *M. soledadinus*, facilitating the examination of short distance interhabitat dispersal on the Kerguelen Islands (Lalouette et al. 2009). All adults of *M. soledadinus* were sampled from under every stone along a 100 m transect line. Microsatellite genotyping demonstrated that this species also exhibited a highly limited genetic diversity along the transect length, suggesting a high rate of short-distance dispersal (Lalouette 2009). This finding is relevant with respect to the step by step colonisation of the habitats by *M. soledadinus*, and consequently high connectivity between the populations in comparison to flight capable species such as aphids and flies. Datasets are currently being compiled to determine the colonisation pathways of *M. soledadinus* at the scale of Kerguelen Islands.

To what extent are native communities' vulnerabilities due to interactions with alien species?

Feeding interactions between native and introduced insects

On the Kerguelen Islands, there are only three native invertebrate predators, a small rove beetle *Antarctophytosus atriceps* (Staphylinidae) and two spiders, *Myro kerguelensis* (Desidae) and *Neomaso antarcticus* (Linyphiidae). Hence, the predatory nature of the introduced *M. soledadinus* and scarcity of competitors and/or predators, means that it is fast becoming a dominant species in arthropod communities on these sub-Antarctic islands (Laparie et al. 2010). As a result, this species presents a major threat to the native invertebrate fauna. For example, the native wingless fly *Anatalanta aptera* has long been considered to be a preferred prey of *M. soledadinus*, based on its disappearance from most of the habitats that have been colonised by this ground beetle (Table 2).

In 2006, one population of *A. aptera* was found at a distance of approximately 300 m from Port Elisabeth (Table 2), a site where this species has been considered as extinct. Therefore, it would be of interest to determine whether this is a temporal recolonisation of the habitat by *A. aptera* individuals,

Table 2 Changes in the distribution of the endemic fly *Anatalanta aptera* and the introduced ground beetle *Merizodus soledadinus* at several sites at the Kerguelen Islands between 1939 and 2010

	Anatalanta aptera	*Merizodus soledadinus*
Port Couvreux		
1939	+	+
1983	−	+
2010	−	+
Port-Elisabeth		
1939	+	+
1983	−	+
2010	±	+
Ile du Port		
1939	+	−
1991	+	+
2010	+	+
Port aux Français		
1983	+	−
1994	+	−
2010	−	+
Pointe Suzanne		
2005	+	−
2010	+	+

+ = presence; − = absence. Updated from Chevrier et al. (1997)

or whether this population has been accidentally missed during previous field observations. Chevrier (1996) and Chevrier et al. (1997) investigated the competitive interaction between *M. soledadinus* and *A. aptera* under field conditions and examined the impact of an increasing number of *M. soledadinus* on the biology of *A. aptera*. It was found that, when equal numbers of *A. aptera* and *M. soledadinus* were present, there was a four fold reduction in the production of *A. aptera* offspring than when *M. soledadinus* was completely absent (i.e. the control). Conversely, the presence of *A. aptera* had no beneficial impact on the number of immature stages of *M. soledadinus*. Under controlled conditions, the adults of *M. soledadinus* were observed to eat both alien and native invertebrate species, including larval instars of *Pringleophaga kerguelensis* (Lepidoptera: Tineidae) that were 80 times heavier than *M. soledadinus*. Even if a switch in prey selection may result in alterations to the morphology of an adult (see Laparie et al. 2010), *M. soledadinus* should be considered as an opportunistic generalist predator. The actual impact of this invasive ground beetle on native biota may have been underestimated by existing studies, and in any case native invertebrates should be considered as highly endangered.

The competitive interaction between the native wingless fly *A. aptera* and the flight-capable alien fly *C. vicina* was also examined. During flight-favourable thermal periods, the high dispersal capability of *C. vicina* adults was found to facilitate faster colonisation and resource exploitation than that of the flightless *A. aptera*. *Calliphora vicina* is also characterised by a shorter development period and a larger offspring than *A. aptera* (Vernon 1981; Davies 2006). Therefore, *C. vicina* can often represent the major part of the larval biomass in carrions (Chevrier et al. 1997). Clear conclusions were not obtained in experimental research on the competitive interaction between *C. vicina* and *A. aptera* (Chevrier et al. 1997). Large amounts of organic matter can be found at littoral habitats (i.e. penguin colonies or elephant seal moulting zones). This resource may become limited during winter or at inland and high altitude areas (i.e. precipitous cliffs) that are devoid of dense vertebrate colonies (with the exception of petrels at some sites) (Vernon 1981). Hence, intraspecific competition could occur for *A. aptera* when carrions were first colonised by the flight capable species *C. vicina*, thereby limiting the availability of suitable habitats (Chevrier et al. 1997). The negative impact of *C. vicina* on *A. aptera* population dynamics may be subject to variation throughout the archipelago, but the parallel predation and competitive pressures of both *M. soledadinus* and *C. vicina* are likely to impair the littoral populations of this species.

Aphids constitute some of the most harmful phytophagous insects of temperate regions (Tatchell 1989). The first aphids were observed on the Kerguelen Islands in 1965, and were probably introduced with the plants that were cultured in the greenhouses (Hullé et al. 2003a). Except for *Myzus persicae* (the most recently introduced aphid in 2000), the precise episodes of introduction were poorly documented. Aphids feed on the phloem sap of plants, and thus weaken plants by causing direct physical damage and/or indirect damage through the transmission of a number of viruses. In fact, aphids transmit over 50% of plant viruses vectored by insects, i.e. 19 virus genera comprising about 275 species (Fereres and Moreno 2009; Nault 1997).

The five aphid species that were introduced to the Kerguelen Islands, particularly *Myzus ascalonicus* and *M. persicae*, are well known to be potential vectors of tens of viruses belonging to different virus families (Blackman and Eastop 2000). In addition, *M. ascalonicus* is the most invasive aphid species on the Kerguelen Islands, having colonised several sites far from the initial site of introduction, including the central and eastern parts of the archipelago. This species has now colonised five native and 21 introduced plants, pertaining to 14 botanical families (Hullé et al. 2003b). *Myzus ascalonicus* is particularly abundant on two native plants, *Leptinella plumosa* and *Acaena magellanica*, and on two introduced plants, *Taraxacum officinale and T. erythrospermum*. The endemic plant *Pringlea antiscorbutica* may also host large colonies of *M. ascalonicus.* Damage caused by this aphid species is primarily evident on *P. antiscorbutica*, *A. magellanica* and *T. officinale*. Plants may be spoiled or even killed as a result of aphid attacks. Damage is often limited to a few individual plants; however, on occasion damage may cover the surfaces of plants in an area of up to one hectare in size. *Myzus ascalonicus* is currently restricted to the first 100 m above sea level, because of a rapid decrease in air temperature with increasing altitude. However, vegetation is abundant at elevations up to 200 m above sea level, hence there is an opportunity for the insect to extend its range in response to warming events (Hullé et al. 2003a). The aphid *Rhopalosiphum padi* has colonised three native and 14 introduced grass species. It was particularly abundant on the native plant *Poa cookii*, and the introduced plants *Holcus lanatus*, *H. mollis* and *Poa annua*. Aphid damage is visibly characterised on *P. cookii* by yellow rings.

Would microscopic and often missed enemies be more harmful to native fauna and flora than alien invertebrates and plants?

Studies of biological invasions have had a tendency to focus on visible species, such as plants, invertebrates and vertebrates, or the impact of invasive colonists on native communities. However, a fact that is frequently overlooked in most studies of biological invasions is that every species of plant and animal is a potential host of bacteria, viruses, protozoa or nematodes. Plant viruses are globally widespread because they can be vectored by phytophagous insects. Hence, as a result of the introduction of plant species on sub-Antarctic islands, it is a logical hypothesis that associated viruses may also have arrived. Based on this assumption, a systematic search for viral infection in both symptomatic and asymptomatic plants (native and alien species) has recently been initiated at the Kerguelen Islands.

To date, there has been just one report describing the presence of a plant virus in a sub-Antarctic environment that of *Stillbocarpa mosaic bacilliform badnavirus* (SMBV) (Skotnicki et al. 2003). In a preliminary study at the Argentina Islands, Polischuk et al. (2007) reported the detection of antigens in the Antarctic hairgrass *Deschampsia antarctica* (a plant species also present on the Crozet, Kerguelen, Heard islands and South Georgia) to three well known and widespread plant viruses, *Cucumber mosaic virus* (CMV, *Cucumovirus*), *Cucumber green mottle mosaic virus* (CGMMV, *Tobamovirus*) and *Tomato spotted wilt virus* (TSWV, *Tospovirus*). Recently, Marais et al. (2010) provided the first report of the presence of the *Cherry leaf roll virus* (CLRV) (*Nepovirus*) on Nasturtium plants on Amsterdam Island. On the Kerguelen Islands, analysis of *Acaena magellanica* specimens has already indicated symptoms similar to viral infection, and hence the likely presence of viruses.

Comparisons of a sequence from cDNA clones, derived from one sample of *A. magellanica*, with viral sequences from databanks, indicated 64% similarity (and 50% identity) with the coat protein of the *Black raspberry virus F*, an unclassified virus for which only limited information is currently available (unpublished data T. Candresse T, C. Couture, C. Faure, A. Marais, L. Svanella-Dumas). It has yet to be determined whether this is a fungal or a phytopathogenic virus. Future studies should examine whether (1) plant viruses were introduced to the Kerguelen Islands with plants and/or vectors (i.e. aphids), (2) viruses have already been transmitted to native plant species through introduced phytophagous insects, and (3) introduced viruses play a role in the invasion success of alien plants as suggested in other plant/virus models (Borer et al. 2007; Gibbs et al. 2008; Malmström et al. 2005, 2006).

What have we learnt from the sub-Antarctic islands?

Insular ecosystems, such as the sub-Antarctic islands, provide an “ideal laboratory” to address the problems associated with harmful invasive species, including animals, plants and viruses. While these findings are constrained to a specific environment, the insights obtained on the colonisation mechanisms, genetic fitness and impact on native species can be subsequently applied to more complex temperate continental systems, particularly in the context of climate change. The geographical isolation of Antarctica and the sub-Antarctic islands is diminishing due to an ever increasing number of humans visiting this region providing the opportunity for invasive species to take hold. Furthermore, recent changes in both precipitation and temperature levels have been shown to facilitate the establishment of these aliens not only on the sub-Antarctic islands, such as the Kerguelen Islands, but also on the Antarctic Peninsula itself. For instance, the invasive annual meadow-grass *Poa annua* (Poaceae) can already be found at several locations on both sub-Antarctic and Antarctic islands (Convey et al. 2006). The sub-Antarctic islands provide quantitative clarification of the threat posed by alien species to extreme, insular environments.

These findings should be used to develop a range of mitigating measures for incorporation into existing guidelines, such as the Madrid Protocol (Antarctic Treaty Consultative Meeting 2003). We will maintain what is left of the biodiversity of the Antarctica and the sub-Antarctic islands only through minimising opportunities for the establishment of alien species.

Acknowledgments We are very grateful to Dr. Jean-François Voisin for helpful discussions concerning the distribution of aphids in the early 1960s and to Jean-Christophe Simon for valuable information on the genetics of *Rhopalosiphum padi*. The authors thank Dr. Gail Schofield and Dr. Jonathan Wilson for helpful amendments on a previous version of our manuscript. This research was supported by the "Institut Polaire Francais" (IPEV 136), the CNRS (Zone Atelier de Recherches sur l'Environnement Antarctique et Subantarctique) and the "Agence Nationale de la Recherche" (ANR-07-VULN-004, EVINCE). This research is linked with the SCAR *Evolution and Biodiversity in the Antarctic* research programme. Mathieu Laparie is supported by a fellow from the MENRT (Université de Rennes 1, France).

1.3.3. Organisation et structure de la thèse

Cette thèse s'inscrit dans le programme 136 ÉCOBIO soutenu par l'IPEV (Institut polaire français Paul-Émile Victor), intitulé *Changements climatiques, actions anthropiques et biodiversité des écosystèmes terrestres subantarctiques*, ainsi que dans la Zone Atelier de *Recherches sur l'Environnement Antarctique et Subantarctique*. Les objectifs de ce travail rencontrent ceux de l'ANR EVINCE intitulée *Vulnerability of native communities to invasive insects and climate change in subantarctic islands*, dont les axes fédérateurs ont été présentés dans l'**ARTICLE I**. À travers les cas précédemment détaillés de *M. soledadinus et C. vicina* dans les îles Kerguelen, cette thèse vise à mieux appréhender les paramètres évolutifs et écologiques qui sont étroitement liés au processus d'invasion biologique et au succès des espèces invasives. La réalisation de ces objectifs passe par une meilleure compréhension de la biologie des espèces invasives dans leur nouvel environnement, dont les conditions biotiques et abiotiques se distinguent plus ou moins fortement des conditions propres à l'aire native. Un intérêt tout particulier sera donc accordé aux réponses physiologiques ou morphologiques développées par ces organismes dans la gamme d'environnements envahis et au regard des variations de conditions biotiques (interactions trophiques notamment) ou abiotiques (température, salinité). La réponse à ses problématiques doit par ailleurs permettre de clarifier les processus de dispersion des espèces introduites.

Le **chapitre 2** sera ainsi consacré aux indices morphologiques des nouvelles contraintes que les espèces rencontrent dans leur zone de naturalisation et qui peuvent altérer leurs histoires de vie. Ces variations morphologiques interviennent à travers le temps et l'espace et peuvent refléter à la fois l'adaptation locale rapide et la plasticité phénotypique, ces deux processus étant fréquemment considérés comme particulièrement déterminants dans le succès des invasions (mais leur identification est souvent difficile dans la nature ; *cf.* **partie 2.4.**). Qu'elles soient intergénérationnelles ou plastiques, ces modifications constituent des réponses au régime de sélection du nouveau milieu, différent de celui du milieu natif (**ARTICLE II**), mais peuvent également illustrer l'altération progressive des conditions environnementales que l'espèce invasive provoque elle-même en perturbant l'écosystème (**ARTICLE III**). L'hypothèse alternative de la sélection de phénotypes disperseurs aux marges du front de colonisation sera également abordée dans ce chapitre (**ARTICLE IV**).

Après une caractérisation de la variété des microhabitats dans lesquels se rencontre préférentiellement *M. soledadinus* aux Îles Kerguelen, le **chapitre 3** sera focalisé sur le rôle primordial de la plasticité dans le succès invasif de cette espèce. Les ajustements physiologiques

(**ARTICLE V**) et trophiques (**ARTICLE VI**) seront abordés pour illustrer les mécanismes participant à la colonisation et la persistance dans de nouveaux environnements, différents de l'environnement natif, ou progressivement perturbés par l'invasion elle-même. Nous aborderons par ailleurs l'exemple d'une espèce native qui, par sa relation réciproque avec les invasives, se trouve confrontée à des perturbations écologiques et doit répondre rapidement aux changements de pressions sélectives (**ARTICLE VII**), la plasticité phénotypique étant souvent vue comme un paramètre capital de la réponse rapide aux variations de l'environnement.

CHAPTER 2

MORPHOLOGICAL CHANGES IN TIME AND SPACE IN NATURALIZED SPECIES

The form, then, of any portion of matter, whether it be living or dead, and the changes of form which are apparent in its movements and in its growth, may in all cases alike be described as due to the action of forces.

— D'Arcy Wentworth Thompson (*On Growth and Form*, 1917)

2.1. Novel selection regimes: a flyer in islands where flightlessness is the rule (ARTICLE II)

After introduction, alien species encounter novel selection regimes characteristic of their new environments (Facon et al. 2006). In sub-Antarctic Islands, the striking evolutionary convergence of flightless insects highlights strong selection pressures on flight dispersal (see Schermann-Legionnet et al. 2007, Roderick & Vernon 2009). The invasion of *C. vicina* at the Kerguelen Islands thus provides an interesting case study for investigating how invasive species fit the new conditions, as flight is a key feature of the life strategy of this species known as a strong disperser (Williams & Richardson 1983, Davies 2006). In the following study, wing size and shape of *C. vicina* from the Kerguelen Island and from a native control population were studied using geometric morphometrics. We studied the nature and the rate of the phenotypic changes that occurred over time in both populations. As global warming is thought to be the factor that enabled the full development of *C. vicina* at the Kerguelen Islands (ARTICLE I), at its thermal margins, we also examined the thermal reaction norms of wing morphology in individuals from both populations reared at the laboratory. The possible relationship between actual changes observed in the field and thermal plasticity of wing phenotype is discussed.

Laparie, Vernon *et al. in prep.* Article II

When aliens meet novel selection pressures: morphological change of the wing in a flying insect invading a world of fligthlessness

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Abstract

The cosmopolitan blowfly *Calliphora vicina* was naturalized at the sub-Antarctic Kerguelen Islands in the late 1970s, following a warming period allowing its full development. Although temperature and wind may limit its flight activity, it invaded the archipelago towards sites remote from the introduction point. Most native food-competitors have converged to flightlessness under the sub-Antarctic conditions, and the flight ability of *C. vicina* might be either a handicap or a competitive advantage with regard to ongoing climate change. Using geometric morphometrics, we questioned whether the wing changed over time within the archipelago (1998 vs. 2009) and this was compared to a continental population from the native range (1983 vs. 2009). Variations after development at different temperatures in controlled conditions were also investigated in both populations. We found different reaction norms to temperature for wing size and shape between the populations, with higher magnitude for shape in individuals from Kerguelen. The highest allochronic change in wing shape was also found in females from Kerguelen, despite a shorter time-lag between the samples compared to the continental population. Our findings demonstrate rapid changes of the wing of *C. vicina* at its margins. From an evolutionary standpoint, these changes, alongside phenotypic plasticity, are of interest given the flightlessness rule generally observed in native sub-Antarctic insects.

Keywords

Allochronic change, geometric morphometrics, invasive insect, local adaptation, phenotypic plasticity, sub-Antarctic islands.

Laparie, Vernon *et al. in prep.* Article II

Introduction

Wings are key adaptations that have facilitated the diversification of insects. Insect wings are subject to strong canalization (Gilchrist and Partridge 2001), which is a measure of the ability to produce a consistent phenotype despite genetic and environmental impacts (Debat and David 2001; Debat et al. 2009). One of many functions of wings (ranging from thermal regulation to camouflage) is active flight, which is a rapid and energy efficient mode of dispersal (Dickinson et al. 1999; Dudley 2002). The evolution of flight ability improved several features in insect ecology, including mating, survival (*e.g.*, rapid escape from predators), and access to distant ecological resources (Dickinson et al. 1999; Dudley 2002). Dudley (2002) emphasized the role of flight in triggering the adaptive radiation of insects into many ecological roles, including herbivores, pollinators, blood feeders, and long range dispersers; thus contributing to the extreme diversity of winged insects (or pterygotes). In some instances, however, the occupied physical environment may prevent flight or render long-range dispersal useless (*e.g.*, subterranean insects). As a result, wing reduction or winglessness is found in many species, presumably resulting from the counter-selection of wing development or from trade-offs with other biological functions (Roff 1986; 1990).

The example of insects from the sub-Antarctic Islands is particularly striking. Within these islands, the Kerguelen Islands are among the most remote terrestrial habitats worldwide, with Africa being the closest continent (after Antarctica), which is 4110 km away (Chown et al. 1998). Schermann-Legionnet et al. (2007) showed that 83% of the 23 native insects on the Kerguelen Islands have evolved flightlessness or even winglessness. It includes native brachyceran flies such as *Anatalanta aptera* (Diptera, Sphaeroceridae), *Calycopteryx moseleyi* (Diptera, Micropezidae), *Amalopteryx maritima* (Diptera, Ephydridae), *Apetenus litoralis* (Diptera, Coelopidae), nematoceran flies such as *Halirytus amphibius* (Diptera, Chironomidae), and even insects from other orders such as the moth *Pringleophaga kerguelensis* (Lepidoptera, Tineidae) (Schermann-Legionnet et al. 2007). These authors also reported that the only native winged species are weak fliers, such as the polymorphous fly *Listriomastax litorea* (Diptera, Tethinidae), which has three morphs but only one compatible with flight (Séguy 1965). This evolutionary convergence in sub-Antarctic insects has been associated with several closely combined environmental factors (Vernon 1981). First, flying activity requires high metabolic rate that may not be met under the year-round cool temperatures and buffered climate of the sub-Antarctic islands. Second, the strong winds encountered in these areas (Vernon 1981; Chevrier 1996) may shorten possible flight windows (see Taylor 1963). Third, the selective pressures for long-range dispersal may be lowered in native saprophagous invertebrates, which represent the dominant feeding guild (Lebouvier et al. 2011). In fact, these insects mostly complete their development along the coast, as a result of large amounts of trophic resources supplied by the breeding colonies of sea vertebrates (seabirds, penguins, seals) each year (Vernon; 1981; Vernon et al. 1998). Fourth, the selective pressure for rapid locomotion and escape abilities may have been reduced over time as a consequence of very low native predation pressures in invertebrate communities (Chevrier et al. 1997; Laparie et al. 2010; Lebouvier et al. 2011). To conclude, the benefits of wing development and flight may have been counterbalanced by the associated costs, thus promoting the selection of new life strategies based on flightlessness or winglessness. As an example, imagos of *A. aptera* even lost flight muscles, providing space for increased thoracic reserves; thus enhancing starvation resistance during austral winters, and enabling individuals to remain active up to six months without food (Vernon 1981; 1986).

Recent human activities at the Kerguelen Islands have increased the diversity of insect trophic guilds, as well as reproductive and dispersal strategies (see Chown et al. 1998; Lebouvier et al. 2011). The introduction of species to the Kerguelen Islands has long been impeded due to (i) the geographic and climatic isolation, and (ii) low levels of human visitation (the archipelago was discovered in 1772, it has no permanent inhabitants nor airport facilitating visitation) (Chown et al. 1998; Frenot et al. 2005; Lebouvier et al. 2011). Many alien introductions occurred as a result of past whaling activities and the development of scientific research since the 1950s (Jeannel 1940; Frenot et al. 2001; Frenot et al. 2005; Chown et al. 2008). Frenot et al. (2005) reported 26 species of naturalized arthropods at the Kerguelen Islands, of which six became invasive. As non-indigenous species must adjust to new abiotic (*e.g.*, climatic factors) and biotic (*e.g.*, species interactions) conditions to successfully establish in new habitats (Huey et al. 2005; Facon et al. 2006; Falk-Petersen et al. 2006; Davis 2009), the question is raised of how these invaders adapted to the distinctive conditions of sub-Antarctic islands.

The invasion of the blue blowfly *Calliphora vicina* (Diptera, Calliphoridae) at the Kerguelen Islands offers a unique opportunity to address real time changes in ecological and evolutionary processes (Chevrier et al. 1997; Huey et al. 2005; Sax et al. 2007; Lebouvier et al. 2011). The naturalization of this active flyer was reported in 1978 at the research station on the Kerguelen Islands. It was probably transported by ship and subsequently benefited from warming air temperatures in the 1970s (Chevrier et al. 1997; Lebouvier et al. 2011). The biological characteristics of this invader make it particularly interesting to monitor in this environment. First, *C. vicina* has a saprophagous diet, like native flies. Second, it is the best active flyer on the Kerguelen Islands, and has become one of the most common fully-winged food-competitors of native flies (Schermann-Legionnet et al. 2007). Interestingly, its long-range flight dispersal and *r* strategy represent a new life strategy within the feeding guild of decomposers on these islands (Chevrier et al. 1997). Hence, because the long-term evolution of native flies has led to flightlessness, there is consequently no competitor to match the ability of the blue blowfly to rapidly reach food patches on the Kerguelen Islands.

In this paper, we use landmark-based geometric morphometrics to investigate whether the wing structure of *C. vicina* has incurred changes over time in the particular environmental conditions of the invaded area. As local thermal conditions are close to the lower developmental threshold of *C. vicina* throughout the year (Lebouvier et al. 2011), we also investigated thermal phenotypic plasticity of the wing structure. To address the first question, we compared the population from the Kerguelen Islands to a continental population from a native area (Brittany in France). We compared the wings of individuals trapped in 2009 at the two grographic locations to that of previous records (1998 in Kerguelen and 1983 in Brittany). To address the second question, the broods of individuals trapped at both locations in 2009 were reared under a range of developmental temperatures (from 4 to 20 °C). We hypothesized that: (i) the wing morphology changed quickly over time at the Kerguelen Islands compared to a population from the native range (see the significance of examining evolutionary rates in Hendry and Kinnison 1999), and (ii) the populations produce different reaction norms to temperature.

Material and methods

Sample collection

As a part of a long-term monitoring program conducted at the Kerguelen Islands, wild adults of *C. vicina* were sampled in the vicinity of the Port-aux-Français research station (Kerguelen Islands, France, 49° 21' 00'' S, 70° 13' 00'' E) during the austral summers of 1998 (February and March) and 2009 (January and February). Individuals of *C. vicina* from a native area were trapped in the vicinity of the Paimpont research station (Brittany, metropolitan France, 48° 00' 13'' N, 2° 13' 42'' W) during the summers of 1983 (June and July) and 2009 (August). All flies were collected using Upton-type traps and stored in 70% ethanol. Monthly mean shade temperatures are detailed in Table 1. For each population, we grouped the collections when the trapping sessions lasted two months (*i.e.*, February and March 1998 as well as January and February 2009 at the Kerguelen Islands, and June and July 1983 in Brittany). There was a gap of 11 years between the two trapping sessions at the Kerguelen Islands vs. 26 years in Brittany.

Thermal phenotypic plasticity

At both locations, the individuals trapped in 2009 were reared in plastic boxes, and supplied with pieces of sheep liver *ad libitum* to stimulate reproduction and egg-laying. After egg-laying, adults were immediately placed in 70° ethanol before measurements. Batches of 30 eggs were randomly placed onto *ad libitum* pieces of sheep liver in plastic boxes. Each box was then directly transferred to incubators at one of five temperatures: 4, 8, 12, 16 and 20 °C (± 0.5 °C). Hatching did not occur at 4 °C, but this temperature is close to the lower developmental limit in *C. vicina* (Vinogradova 1991; Davies and Ratcliffe 1994). After completion of development, emergence, and wing opening, the resultant adults reared at 8, 12, 16 and 20 °C were stored in 70% ethanol.

Table 1. Detail of monthly mean shade temperatures for all trapping sessions (source: Météo France data). Mean: average temperature computed from the daily means; Min: average temperature computed from the daily minima; Max: average temperature computed from the daily maxima. The standard error is mentioned between brackets.

Population	Trapping session	Min	Max	Mean
Kerguelen	February 1998 – Past	5.4 (0.4) °C	11.9 (0.6) °C	8.1 (0.5) °C
	March 1998 – Past	4.0 (0.4) °C	11.3 (0.5) °C	7.4 (0.4) °C
	January 2009 – Recent	3.8 (0.3) °C	10.3 (0.5) °C	6.7 (0.3) °C
	February 2009 – Recent	4.8 (0.4) °C	11.9 (0.5) °C	8.1 (0.4) °C
Brittany	June 1983 – Past	9.2 (0.6) °C	20.8 (0.8) °C	15.0 (0.8) °C
	July 1983 – Past	14.5 (0.5) °C	26.0 (0.8) °C	20.2 (0.7) °C
	August 2009 – Recent	11.4 (0.6) °C	23.2 (0.6) °C	17.3 (0.4) °C

Table 2. Sample sizes of the groups analyzed. Individuals reared at the four controlled temperatures are the first offspring of the individuals trapped in the field in 2009 (“Recent” column). “Past” samples correspond to 1998 for Kerguelen and to 1983 for Brittany.

Population	Gender	8 °C	12 °C	16 °C	20 °C	Past	Recent
Kerguelen	Males	40	45	28	40	60	26
	Females	40	44	30	40	59	56
Brittany	Males	20	20	20	20	30	20
	Females	20	20	20	20	29	39

Data acquisition

The left wings were mounted in Euparal on microscope slides. A total of 786 individuals were analyzed (Table 2).

A picture of the ventral side of the wing was captured using a 720 × 576 pixels digital camera connected to a binocular microscope. A set of 14 landmarks per wing was digitized (Fig. 1) with a theoretical precision of 8.8 µm using the TPSdig2™ software by F. J. Rohlf.

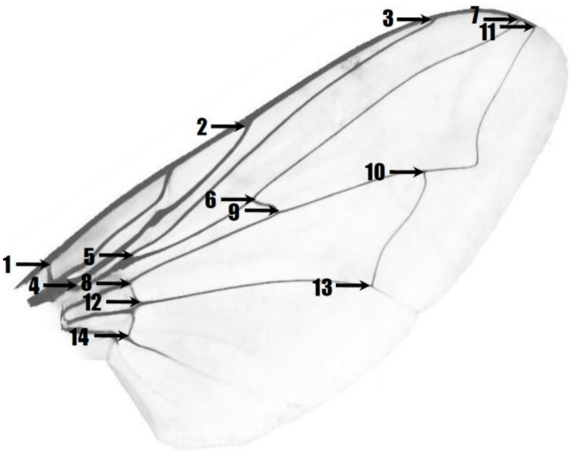

Figure 1. Position of the 14 landmarks digitized on the ventral face of the left wing of *Calliphora vicina*.

Morphometrics and statistical treatment

The landmark configurations were superimposed using a Generalized Procrustes Analysis (GPA; Rohlf and Slice 1990; Dryden and Mardia 1998; Claude 2008). Because the Procrustes superimposition constrains the data (four degrees of freedom are lost resulting in a reduction of dimensionality), Principal Component Analysis (PCA) was applied to the resulting Procrustes coordinates, and the scores on the non-null Principal Components (PC) were used as shape variables in all subsequent analyses (Debat et al. 2009). We used the centroid size as a measure of wing size (see Claude 2008).

Measurement error

Image acquisition and landmark digitization are the two main sources of measurement error in geometric morphometrics (Arnqvist and Martensson 1998). To assess their impact, we applied a design adapted from Alibert et al. (2001) to a subsample of 29 wings. Each wing was photographed, digitized, and in an independent session 45 days later, it was repositioned, photographed and digitized again by the same operator. This method combines the effects of image acquisition and landmark digitization, therefore allowing quantifying the overall measurement error relative to the biological variation among individuals. The statistical significance of measurement error was tested using "individual wing" as the main effect in a Procrustes ANOVA (Klingenberg and McIntyre 1998; Klingenberg et al. 2002).

Size variation

We first tested whether the centroid size was altered by the rearing conditions of the laboratory. We thus compared wild and laboratory-reared individuals in a two-way ANOVA (factors: origin [wild or laboratory], gender, and interaction of the two). We also questioned whether wing size changed (i) over time in wild individuals (hereafter referred to as "allochronic change"), and (ii) depending on the developmental temperature in individuals reared under controlled conditions. In both cases, interaction terms were used to compare wing size variation between populations. This was tested using two separate three-way ANOVAS: (i) trapping year [past or recent] × gender × population, and (ii) temperature × gender × population.

Shape variation

Allochronic change was tested in both genders using MANOVAS (trapping year × population). Four separate Hotelling t^2 tests were then completed for males from Kerguelen, males from Brittany, females from Kerguelen, and females from Brittany, to perform pairwise comparisons between past and recent samples. To reconstruct shape changes per gender within each population, we used four discriminant analyses maximizing the separation between the past and recent samples to 100% of inertia along the LD1. For each analysis, the Procrustes distance between the past and recent groups was calculated. As previous MANOVAS resulted in inter-population differences in allochronic changes in females only, we further focused on this gender. All wild females from both populations were compared in a discriminant analysis to examine the nature of these changes.

Thermal phenotypic plasticity was investigated in both genders using MANOVAS (temperature × population) and was further investigated in a discriminant analysis using PC scores.

Matching phenotypic plasticity and allochronic change

Allochronic and thermal shape changes were compared between the populations using Principal Coordinates Analysis (PCO, also known as metric multidimensional scaling; Mardia et al. 1979; Debat et al. 2006; 2009; Mitteroecker and Bookstein 2009). This analysis is related to Principal Components Analysis, but allows the similarity among a set of matrices to be displayed. Because phenotypic shape changes were non-linear across the thermal range, no single shape axis could be plotted against temperature. We thus estimated the shape change for each pair of consecutive temperatures separately (8–12, 12–16 and 16–20 °C), resulting in vectors of shape changes instead of matrices. For each population, we computed the covariance vectors that depict the different components of shape variation, such as temperature (three vectors per population) and allochronic change (one vector per population). Thus, we obtained eight vectors of shape changes that each corresponded to a single PC (accounting for 100% of the differences in each pair), which were compared in the PCO.

The Procrustes ANOVA was performed using *MorphoJ*™ 1.02c (Klingenberg 2011). All other statistical procedures were conducted with *R* 2.7.2 statistical software (R Development Core Team 2008) using a pre-release of the *Rmorph* library (M. Baylac, personal communication) and the code provided in Claude (2008).

Results

Measurement error

We tested the measurement error by mixing the effects of image acquisition and digitization in a Procrustes ANOVA. The possible effect of time between measurements was taken into account by measuring the same wings in two sessions separated by a period of 45 days. This procedure allowed the overall measurement error to be quantified relative to the biological variation among individuals. The impact of relative measurement error on centroid size and shape was found to be extremely low, as it was 1095.6 and 41.6 times lower, respectively, than the variation resulting from biological inter-individual variation (Table 3).

Change in wing size

When considering the whole dataset, the wing was significantly larger in females than in males, and larger and less variable in laboratory-reared flies than in wild flies (Table 4, Fig. 2). Sexual dimorphism was differentially expressed in wild and in laboratory-reared flies, as demonstrated by the significant interaction between gender and origin (Table 4), mostly because sexual dimorphism for wing size was not significant in wild flies from the Kerguelen Islands (Tukey *post-hoc* tests not shown). Wild and laboratory-reared individuals were further analyzed separately (Table 5).

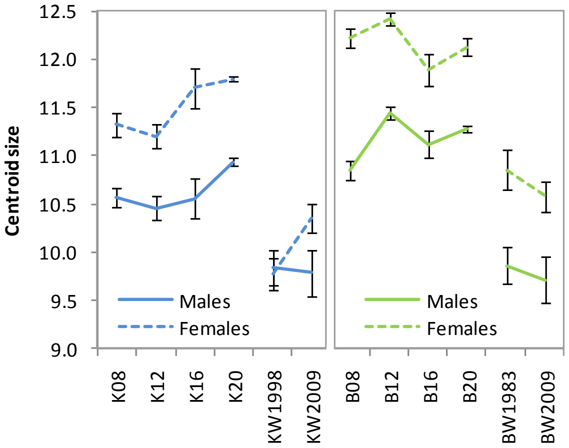

Figure 2. Interaction plot of mean centroid sizes per group. Left frame, blue lines: Kerguelen Islands. Right frame, green lines: Brittany. Legend: "K08–K20" for Kerguelen flies reared at 8–20 °C; "KW1998–KW2009" for Kerguelen Islands wild flies; "P08–20" for Brittany flies reared at 8–20 °C; "BW1983–BW2009" for Brittany wild flies. Error bars: standard error.

The wing size of wild individuals did not significantly evolve within the time-frame of our analysis (Table 5, Fig. 2). The non-significance of all interactions involving allochronic change demonstrated the reliability of this result, whatever the gender and population considered. However, in females from the Kerguelen Islands, the wing tended to increase in size over time. On average, the wings were larger in Brittany.

Table 3. Results of the Procrustes ANOVA computed on individuals photographed and digitized twice for assessing measurement error. The *F* statistic is the ratio between the variation due to interindividual differences and the variation induced by measurement error. All numeric data are rounded to one decimal digit. Significance code: < *0.0001* "***"

Dataset	Effect	SS	MS	df	*F*	*P*
Centroid size	Individual	83.8	3.0	28	1095.6	***
(*N* = 29*2 = 68 digitizations)	Error	$7.9 . 10^{-2}$	$2.7 . 10^{-3}$			
Shape	Individual	$1.5 . 10^{-2}$	$2.2 . 10^{-5}$	672	41.6	***
(*N* = 29*2 = 68 digitizations)	Error	$3.7 . 10^{-4}$	$5.2 . 10^{-7}$	696		

Table 4. Results of the global two-way ANOVA computed on the centroid size. All numeric data are rounded to one decimal digit. Significance codes: *< 0.001* "***"; *< 0.01* "**"

Dataset	Effect	F_{df}	SS	MS	*P*
All individuals	Gender	90.6_1	81.4	81.4	***
(*N* = 786)	Origin	318.6_1	286.1	286.1	***
	Gender:Origin	9.2_1	8.2	8.2	**

Table 5. Results of the two ANOVAs computed on the centroid size for individuals trapped in the field and individuals reared at the laboratory. All numeric data are rounded to one decimal digit. Significance codes: *< 0.001* "***"; *< 0.01* "**"; *< 0.05* "*"; not significant "*n. s.*"

Dataset	Effect	F_{df}	SS	MS	*P*
Wild individuals	Allochronic change	3.2_1	4.3	4.3	*n. s.*
(*N* = 319)	Gender	11.5_1	15.4	15.4	***
	Population	6.7_1	9.0	9.0	*
	Allochronic change:Gender	2.4_1	3.2	3.2	*n. s.*
	Allochronic change:Population	2.4_1	3.3	3.3	*n. s.*
	Gender:Population	6.8_1	9.2	9.2	**
	Allochronic change:Gender:Population	1.9_1	2.5	2.5	*n. s.*
Laboratory-reared individuals	Temperature	6.8_3	8.7	2.9	***
(*N* = 467)	Gender	220.3_1	94.3	94.3	***
	Population	90.8_1	38.9	38.9	***
	Temperature:Gender	0.5_3	0.6	0.2	*n. s.*
	Temperature:Population	7.7_3	9.9	3.3	***
	Gender:Population	1.0_1	0.4	0.4	*n. s.*
	Temperature:Gender:Population	2.5_3	3.2	1.0	*n. s.*

Interestingly, the slopes from 8 to 20 °C were positive in flies from the Kerguelen Islands, with the largest wing sizes being observed after development at the warmest temperature. This pattern was not found in flies from Brittany, as shown by the significant interaction between temperature and population. Overall, sexual dimorphism was maintained over the thermal range regardless of population, as demonstrated by the non-significance of all interactions involving gender.

Allochronic change of wing shape

Significant allochronic changes in wing shape were detected for both genders (Table 6). These shape changes were similar for males from both Kerguelen Islands and Brittany, as shown by the non-significant interaction term. Conversely, the change in wing shape was different for females from Kerguelen and Brittany.

The allochronic shape changes per gender within each population were reconstructed using pairwise discriminant analyses, each separating past and recent samples along LD1s (100% of inertia; Fig. 3). These results confirmed the stronger changes that have been previously found in Kerguelen, especially in females. Landmarks shifts were found mostly in the posterior-distal region of the wing of females from the Kerguelen

Table 6. Results of the two MANOVAs computed on the PC scores for males and females trapped on the field. All numeric data are rounded to one decimal digit. Pillai trace is multiplied by 10. Significance codes: < *0.001* "***"; < *0.01* "**"; < *0.05* "*"; not significant "*n. s.*"

Dataset	Effect	F_{df}	Pillai	*P*
Wild males	Allochronic change	4.4_{24}	4.9	***
(*N* = 136)	Population	8.9_{24}	6.6	***
	Allochronic change:Population	1.6_{24}	2.6	*n. s.*
Wild females	Allochronic change	5.6_{24}	4.6	***
(*N* = 183)	Population	11.7_{24}	6.4	***
	Allochronic change:Population	2.9_{24}	3.1	***

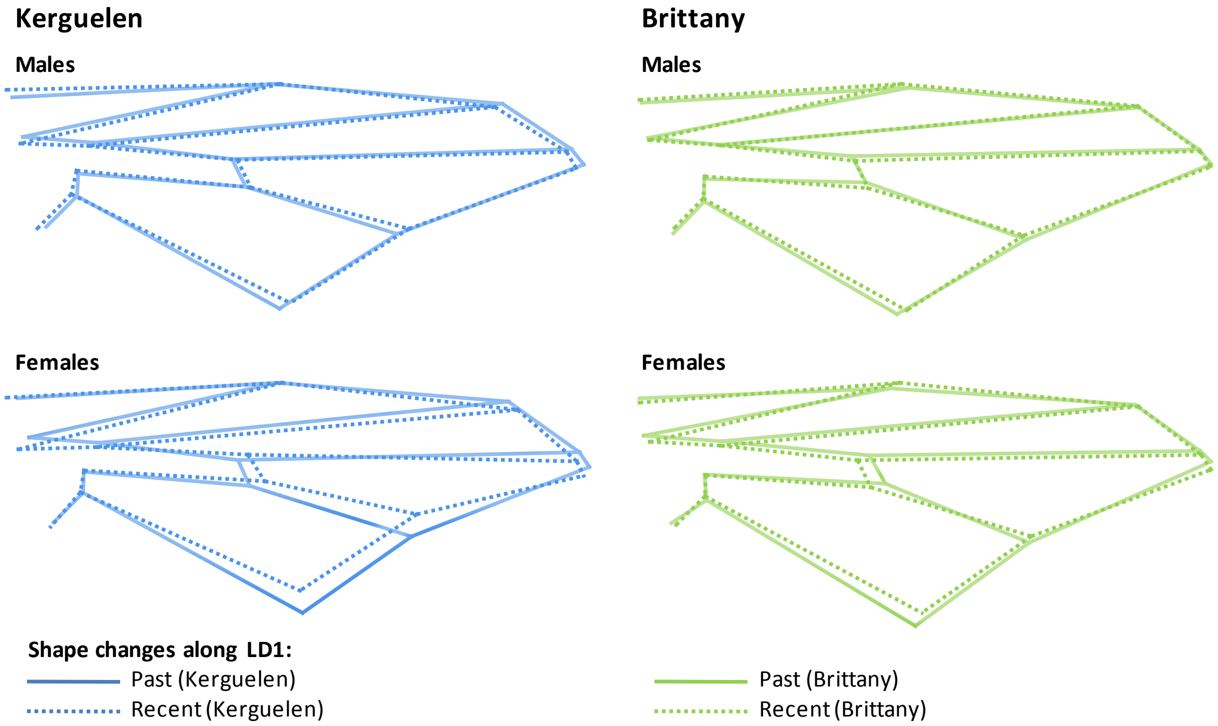

Figure 3. Allochronic shape change within each population and gender, reconstructed from separate discriminant analyses (Kerguelen: 19982009; Brittany: 1983-2009). The changes from past to recent are amplified and correspond to a 15 unit change along LD1s (accounting for 100% of the inertia).

Islands. The crossvein shifted location over time in females from both populations, but in opposite directions.

The most significant allochronic change occurred in females from the Kerguelen Islands, as demonstrated by the Hotelling t_2 tests that were performed to check the statistical significance of the variations illustrated in Fig. 5 (Table 7). The second highest change was found in males also from Kerguelen (see the Procrustes distances between past and recent samples, the *F* statistics and significance levels; Table 7). Of additional note, the time lag between the sampling sessions was shorter in this population than in Brittany (11 vs. 26 years), and the number of generations per year is much lower (from one to two only).

Table 7. Results of the four Hotelling t^2 computed on the PC scores to test the allochronic effect in wild individuals. All numeric data are rounded to one decimal digit. Procrustes distance is multiplied by 10^3. Significance codes: < *0.001* "***"; < *0.01* "**"

Dataset	Effect	F_{df}	Hotelling	Procrustes distance	*P*
Wild males (Kerguelen, *N* = 86)	Allochronic change	3.8_{24}	1.5	6.7	***
Wild males (Brittany, *N* = 50)	Allochronic change	3.5_{24}	3.4	5.8	**
Wild females (Kerguelen, *N* = 115)	Allochronic change	6.8_{24}	1.8	10.4	***
Wild females (Brittany, *N* = 68)	Allochronic change	2.3_{24}	1.3	5.9	**

Because the allochronic change was strong and different in females from the two populations, it was further investigated using discriminant analysis (Fig. 5). The analysis significantly separated the four groups (LD1: *Wilks λ* = 0.143, *p* < 0.001; LD2: *Wilks λ* = 0.418, *p* < 0.001): the first axis, which accounted for 62.2% of inertia, mainly corresponded to a population axis (Fig. 4A). The second axis, which accounted for 30.4% of inertia, corresponded to an allochronic axis. The morphological differences between the two populations implied changes in several components of the wing, including the anterior-proximal and the posterior-distal regions. The crossvein did not appear as a discriminant feature for separating the females from Kerguelen and Brittany. The second axis revealed a striking pattern of allochronic change in the Kerguelen flies (Fig. 4A), whereas only minor variation was found in Brittany (see the bimodal density of individual projections along LD2; Fig. 4B). Consistent with this analysis, the shape changes responsible for the inertia along LD2 were extremely close to the shape changes previously found in females from the Kerguelen Islands only (Fig. 3).

Thermal phenotypic plasticity of wing shape

Wing shape was significantly altered by developmental temperature in both genders of the two populations (Table 8). However, the two populations showed different reaction norms, as illustrated by the significant interaction terms in males and females (Table 8).

Discriminant analysis confirmed that, despite a common general pattern, the thermal reaction norms were different between the Kerguelen Islands and Brittany (Fig. 5). Males mostly differed with thermal changes from 8 to 12 °C (perpendicular vectors). Females differed with thermal changes from 8 to 12 °C and 16 to 20 °C. The magnitude of the thermal reaction norms was the highest in males from the Kerguelen Islands (more variations along LD1, similar variation along LD2, see Fig. 5). Aside from the differences in reaction norms, the analysis revealed strong sexual dimorphism for shape, with the two genders being separated regardless of population or developmental temperature (LD1: *Wilks λ* = 0.001, *p* < 0.001; LD2: *Wilks λ* = 0.008, *p* < 0.001).

Increasing temperature mainly caused the posterior region of the wing to shrink to a more proximal position (LD2), but some temperature changes primarily shifted the crossvein towards a more distal position (LD1). The different shapes of the reaction norm trajectories between populations were consistent with the significant interactions that were previously found (Table 8).

Table 8. Results of the two MANOVAS computed on the PC scores for males and females reared at the laboratory. All numeric data are rounded to one decimal digit. Significance code: < *0.001* "***"

Dataset	Effect	F_{df}	Pillai	*P*
Laboratory-reared males	Temperature	12.4_{72}	1.8	***
(*N* = 233)	Population	17.1_{24}	0.7	***
	Temperature:Population	6.3_{72}	1.3	***
Laboratory-reared females	Temperature	8.7_{72}	1.5	***
(*N* = 234)	Population	18.1_{24}	0.7	***
	Temperature:Population	5.1_{72}	1.1	***

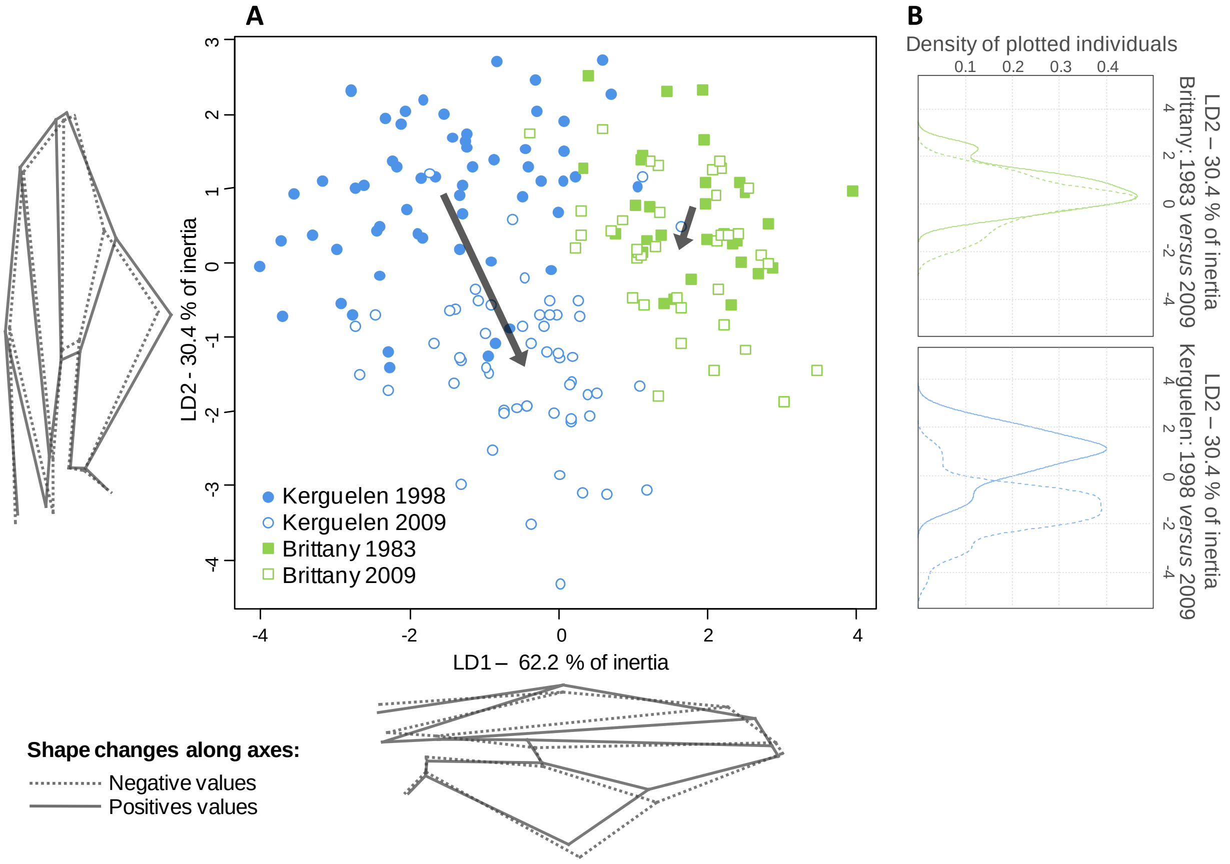

Figure 4. A. Linear discriminant analysis: projection of the 183 wild females grouped by trapping year per population on the first discriminant plane (LD1, LD2). The shape changes are amplified and correspond to a 15 unit change along each axis. Dots: Kerguelen; squares: Brittany; solid symbols: past samples (1998 and 1983 respectively); open symbols: recent samples (2009). The allochronic change within each population is illustrated by the black arrow linking the centroids. The amount of inertia represented by each axis is shown in the axis legend.

B. Density plots of the same individuals along LD2. Note the bimodal distribution in Kerguelen females. Blue: Kerguelen; green: Brittany; solid lines: past individuals; dashed lines: recent individuals.

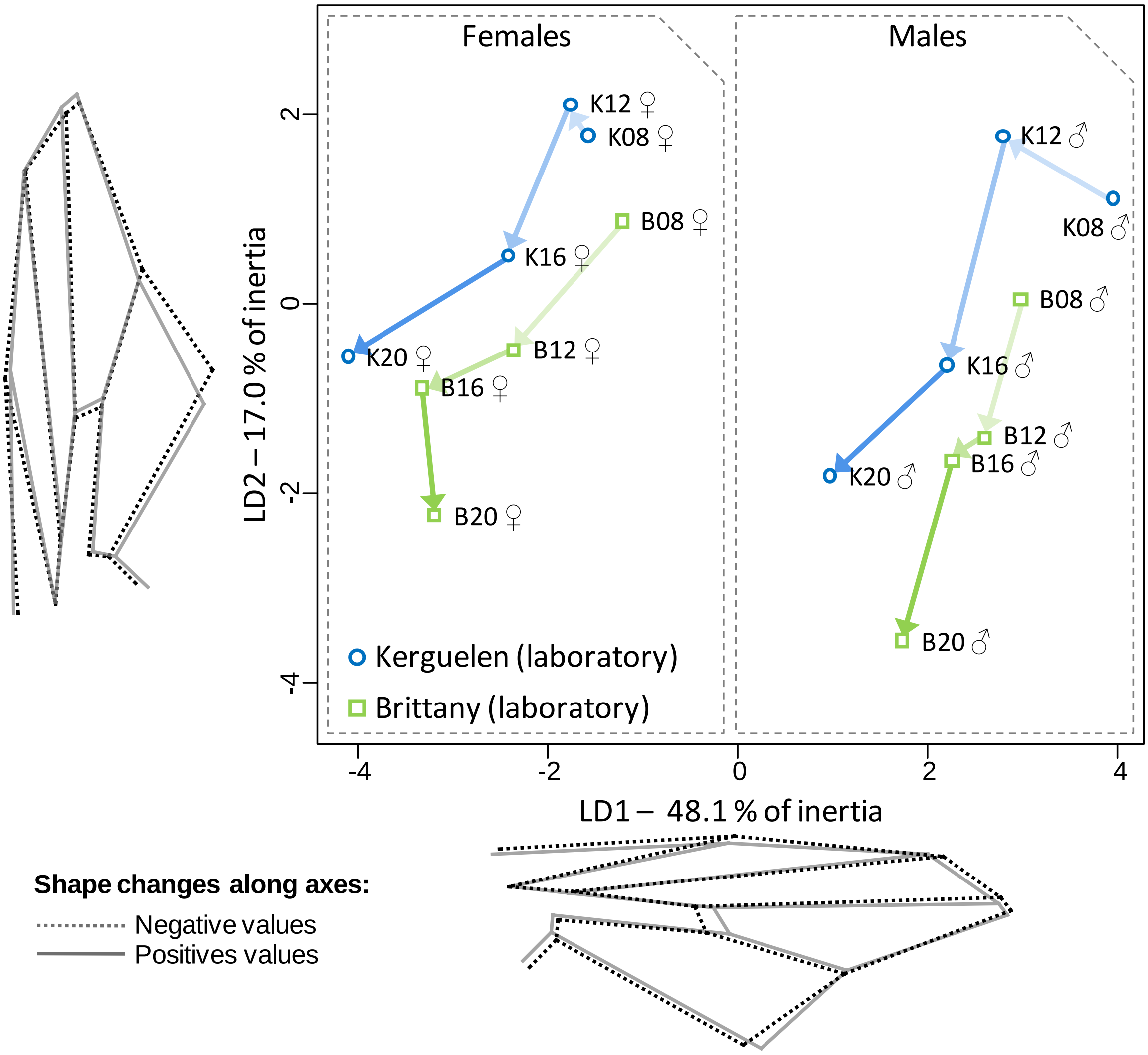

Figure 5. Linear discriminant analysis: projection of the 467 individuals reared at the laboratory on the first discriminant plane (LD1, LD2). Only centroids were plotted for ease of interpretation. The shape changes are amplified and correspond to a 15 unit change along each axis. Dots: Kerguelen; squares: Brittany. The thermal reactions norms within each population are illustrated by the arrows linking the temperature centroids. The amount of inertia represented by each axis is shown in the axis legend.

Linking thermal phenotypic plasticity and allochronic change for wing shape

The angles between vectors depicting each thermal change (08–12, 12–16 and 16–20 °C) and allochronic change (past–recent) were computed for males and females from the two populations. Ordination in a PCO (Fig. 6) confirmed that the two populations produce contrasted thermal reaction norms, despite a cluster between vectors K16–20M, K16–20F and B12–16F. The PCO also highlighted a tight clustering between equivalent thermal changes of males and females from the Kerguelen Islands, as opposed to individuals from Brittany. This result indicates a low level of interaction between thermal plasticity and sexual dimorphism at the Kerguelen Islands.

At the Kerguelen Islands, the allochronic changes in females and males matched the changes observed between 8 and 12 °C (and, to a lesser extent, between 12 and 16 °C), but

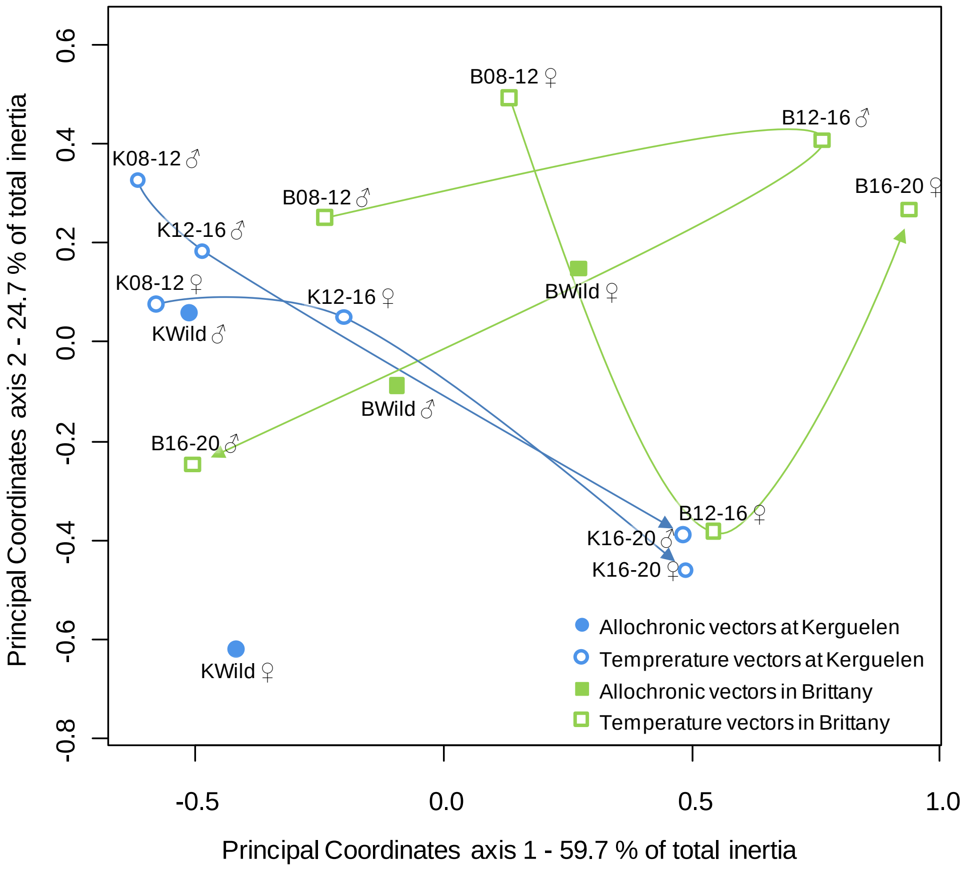

Figure 6. Principal Coordinates Analysis: projection on the first plane of eight shape vectors depicting either the changes induced by temperature shifts at the laboratory or the allochronic changes. Each symbol represents a single matrix. Dots: matrices for Kerguelen; squares: matrices for Brittany; solid symbols: allochronic changes (1983–2009 and 1998–2009); open symbols: temperature shifts. The trajectories through the whole thermal range for each gender of each population are illustrated by the arrows. The amount of inertia represented by each axis is shown in the axis legend.

these females tended to differentiate along the second axis (Fig. 6). A similar association between allochronic changes and thermal changes between 8 and 12 °C tended to appear along the first axis for the flies from Brittany (separately for both genders).

The cosines of angles among vectors allow quantitative pairwise comparisons between the directions of shape changes. These values confirmed the statement of similarity between allochronic changes and thermal changes that is induced at the lowest temperatures for flies from the Kerguelen Islands (Table 9A). Conversely, the cosines also revealed that the association was weak in males from Brittany (Table 9B). Overall, the cosines of angles support the strong similarity between male and female thermal reaction norms at the Kerguelen Islands (Table 9A). In contrast, in Brittany, the similarity decreased from the lowest temperature shift to the higher ones, towards vectors at the highest temperature shift moving in almost opposite directions (Table 9B). Finally, the low values of angles between populations for similar temperature shifts support the above conclusions of contrasted reaction norms (Table 9C). The only vectors that were oriented in similar directions were found for temperature shifts that were not comparable.

Table 9. Cosines of angles between the shape vectors depicting thermal changes (08–16, 12–16 and 16–20) and allochronic change (past–recent) within Kerguelen (A), within Brittany (B), and between the two populations (C). Irrelevant comparisons between thermal changes in one population vs. allochronic change in the other are not shown. Values approaching 1 indicate similar vectors directions (*cos*(0)); values approaching 0 indicate orthogonal vectors directions (*cos*(90)); values approaching -1 indicate opposite vectors directions (*cos*(180)).

A	K08–12 ♀	K08–12 ♂	K12–16 ♀	K12–16 ♂	K16–20 ♀	K16–20 ♂	KWild ♀	KWild ♂
K08–12 ♀	1.0							
K08–12 ♂	0.9	1.0						
K12–16 ♀	0.4	0.2	1.0					
K12–16 ♂	0.6	0.6	0.7	1.0				
K16–20 ♀	-0.2	-0.5	0.1	-0.1	1.0			
K16–20 ♂	-0.2	-0.4	0.3	-0.1	0.9	1.0		
KWild ♀	0.5	0.2	0.3	0.3	0.3	0.3	1.0	
KWild ♂	0.7	0.7	0.3	0.7	-0.1	-0.2	0.5	1.0

B	B08–12 ♀	B08–12 ♂	B12–16 ♀	B12–16 ♂	B16–20 ♀	B16–20 ♂	BWild ♀	BWild ♂
B08–12 ♀	1.0							
B08–12 ♂	0.8	1.0						
B12–16 ♀	-0.1	-0.3	1.0					
B12–16 ♂	0.2	-0.2	0.4	1.0				
B16–20 ♀	0.3	-0.2	0.4	0.6	1.0			
B16–20 ♂	0.1	0.5	0.1	-0.4	-0.7	1.0		
BWild ♀	0.5	0.4	0.3	0.3	0.2	0.3	1.0	
BWild ♂	0.0	0.2	-0.1	-0.1	-0.1	0.2	-0.2	1.0

C	K08–12 ♀	K08–12 ♂	K12–16 ♀	K12–16 ♂	K16–20 ♀	K16–20 ♂	KWild ♀	KWild ♂
B08–12 ♀	0.1	0.0	0.6	0.4	0.0	0.1		
B08–12 ♂	0.4	0.2	0.7	0.7	0.1	0.1		
B12–16 ♀	-0.2	-0.3	0.1	-0.2	0.7	0.7		
B12–16 ♂	-0.4	-0.3	-0.1	-0.3	0.1	0.1		
B16–20 ♀	-0.5	-0.5	-0.3	-0.5	0.3	0.3		
B16–20 ♂	0.7	0.5	0.7	0.8	0.1	0.2		
BWild ♀							-0.1	-0.1
BWild ♂							0.2	0.3

Discussion

Our results support our initial hypothesis that *C. vicina* may face different selection pressures on the wing at the Kerguelen Islands. First, in addition to wing size differences (smaller wings), shape differences in the leading-edge of the wing were related to different functional constraints for the flight (Dickinson et al. 1999; Gilchrist et al. 2000). Second, contrasted thermal reaction norms between the populations are consistent with the idea of local adaptations (Debat et al. 2003; 2008; Loh et al. 2008). Finally, even if phenotypic differences are expected after a bottleneck effect following introduction, or between unrelated populations, a founder effect alone cannot explain the comparatively quick allochronic change observed, especially because the isolation of the Kerguelen Islands reduces subsequent incoming gene flows.

Sexual dimorphism reflects developmental constraints

A relatively steady sexual shape dimorphism, such as that found in the present study for both wing size and shape, is known to reflect morphogenesis and developmental constraints (Gilchrist et al. 2000). In *Drosophila melanogaster* (Diptera, Drosophilidae), Gilchrist et al. (2000) found stable gender-related shape deviations among the studied populations. As reviewed in Fairbairn (1997), the smaller wings of males reflect most descriptions of sexual size dimorphism in insects, despite exceptions existing with respect to taxa, life strategies, and sexual selection. Interestingly, the trend for sexual size dimorphism of the wing was not significant in wild individuals (except between males and females from Brittany 1983; Tukey *post-hoc* tests, $\alpha = 0.05$). Although larger female wings are observed after development under controlled and optimal conditions, they may be hindered under natural conditions. Sexual dimorphism often results from different selection regimes and energy expenditures between genders, with some heavy trade-offs primarily occurring in females due to egg production and dispersal (*i.e.*, locating sexual partners and locating food for offspring) (Williams and Richardson 1983; Davies 2006). Additional genetic and fitness studies are necessary to examine this idea.

Different flight dispersal requirements between invaded and native areas

As emphasized by Hoffmann and Shirriffs (2002), wing shape parameters can be under independent selection from wing size. While size may be subject to directional selection, shape is likely to have been subject to optimizing selection and canalization, resulting, for example, in the conserved wing structure of all Drosophilidae species (Gilchrist and Partridge 2001; Mezey and Houle 2005). Hoffmann et al. (2005) stated that "wing shape is altered more readily under environmental stress than linear measures of fluctuating asymmetry or indices of wing shape asymmetry." Consequently, monitoring variations in wing structure may contribute in the investigation of stressful environmental conditions during development (Hoffmann et al. 2005).

Van Dyck and Matthysen (1999) stated that "evolutionary changes in flight-morphology are symptomatic of populations with altered costs and benefits of dispersal". Evidence of distinct shapes within a species can reflect the coexistence of multiple optimality levels depending on local conditions (Weber 1990; Gilchrist et al. 2000). Such geographic variations in wing shape have been frequently found among distinct populations of *D. melanogaster* (Gilchrist et al. 2000; Gilchrist et al. 2001; Hoffmann and Shirriffs 2002), and are common in cosmopolitan species (Mayr 1963). They often arise as a result of local adaptations to biotic and abiotic factors in particular areas (Ricklefs and Miles 1994). Hence, wing shape may reflect environmental pressures and selection for wing characteristics, as hypothesized for *Drosophila lummei* (Haas and Tolley 1998).

Noticeable differences in wing size and in the anterior-proximal region of the wing were found between females from the two populations. The anterior-proximal region of the wing may be directly impacted by the functional constraints for flight dispersal, as it contributes towards maintaining the axis of rotation near to the leading edge during flight (Dickinson et al. 1999; Gilchrist et al. 2000). This region is usually less variable than the posterior-distal region (see results in Pezzoli et al. 1997; Gilchrist et al. 2000). The smaller wings of individuals from the Kerguelen Islands compared to individuals from Brittany, both in nature and controlled conditions, also support the concept of different cost and benefits for the wing. In two drosophilid species, Loeschcke et al. (1999) reported larger wings at most tested temperatures in species that exhibited the highest dispersal ability in the natural environment.

From an ecological perspective, novel selection pressures on *C. vicina*'s wing in the invaded area are relevant. As emphasized in Medeiros and Gillespie (2011), insect flightlessness is more likely to appear in insular, windswept, and alpine environments, such as the Kerguelen Islands (Le Roux et al. 2002). Hanski (1987) reported that transient habitats – such as carrion in saprophagous species – require colonist individuals to have a high dispersal power. Nevertheless, the main trophic resources for sub-Antarctic decomposers are supplied in vast quantities at coastal areas (Vernon 1981; Lebouvier et al. 2011), thereby reducing the need for high dispersal power, as illustrated in most native saprophagous insects that evolved flightlessness, partly in response to food distribution (Vernon 1981; Hanski 1987; Chevrier 1996). At Port-aux-Français, the coastal area where adults where sampled at the Kerguelen Islands, it is likely that even low powered flight would be sufficient to reach food patches and lay eggs earlier than native competitors during the austral summer. This is of paramount importance, as *C. vicina* is mainly attracted to carrion during the first three days of decomposition (Chevrier et al. 1997).

Our trapping design is common for flies, but is targeted towards capturing active flyers. Due to this potential sampling bias, we cannot exclude the possibility that the genotypes of the least successful flyers were missed here. However, these individuals are more likely to ensure good fitness at the Kerguelen Islands compared to Brittany, given the different modes of distribution of food and mating habitats. In addition, alone, this possible bias could not explain the more significant allochronic change found at Kerguelen.

Allochronic change of the female wing in Kerguelen may reflect life strategy changes

The investigation of phenotypic variation among wild individuals allows a broad range of environmental parameters to be considered, in addition to the certain genetic differences that can be isolated from controlled conditions (Hendry and Kinnison 1999). The wild flies trapped in 1998 and in 2009 at the Kerguelen Islands developed under similar mean temperatures and thermal ranges, thus rejecting the hypothesis that there is a simple direct effect of temperature, and instead supporting that other sources affect variation.

Despite the sampling interval being twice as short compared to Brittany, the allochronic change in wing shape was the most contrasted at the Kerguelen Islands, and specifically in females. The trend of increasing wing size over time that was observed in Kerguelen females also supports an increase in sexual dimorphism over time in the invaded area, mainly resulting from changing female morphology rather than males. These results may reflect life strategies adjustments and higher sensitivity of females to the environmental conditions of the newly colonized area. Morphological alterations primarily expressed in females regarding residence time were suggested in another insect invading the Kerguelen Islands, the ground beetle *Merizodus soledadinus* (Coleoptera, Carabidae) (Laparie et al. 2010).

Many natural pressures may primarily apply in females due to trade-offs opposing energy allocations for reproduction or dispersal (Williams and Richardson 1983; Van Dyck and Matthysen 1999; Davies 2006). Morphological traits that covary with reproductive and dispersal functions are of particular interest in invasive species, as they are prime components of their success in the invaded area (Rosecchi et al. 2001; Chown et al. 2007; Laparie et al. 2010). *Calliphora vicina* is known as a disperser species: the allocation of energy to flight by females of this species is high compared to other saprophagous Calliphoridae, such as *Lucilia sericata* (Williams and Richardson 1983; Davies 2006). However, this comes with a cost in egg production, with *L. sericata* females having a higher fecundity than *C. vicina* females (Williams and Richardson 1983; Davies 2006). Consequently, the comparatively quick change in females from Kerguelen may reflect changes in life strategies that involve dispersal and reproduction traits. The increasing sexual dimorphism at Kerguelen might also reflect changing sexual selection. Indeed, the specific resource availability at Kerguelen (Vernon 1981; Lebouvier et al. 2011) may arrange local breeding systems by influencing the way that individuals are distributed (Lehtonen and Lindström 2004).

Overall, allochronic change involved posterior-distal components that are, to our knowledge, not fully understood as functional constraints for flight (Dickinson et al. 1999; Gilchrist et al. 2000); possibly suggesting the contribution of other life-traits. In the butterfly *Melitaea cinxia* (Lepidoptera, Nymphalidae), Breuker et al. (2007) reported that the covariation between wing shape and dispersal patterns (see also Van Dyck and Matthysen 1999) may be sex-specific. However, they found no difference in the dispersal rates of males and females, despite the overall sexual dimorphism. The investigation of dispersal rates and fecundity across time would potentially provide insights into the covariation of posterior-distal components and life-histories of the populations studied here. Such analyses would allow the concept of evolving trade-offs to be addressed for flight vs. reproduction or other life-traits, as well as to test the sex-specificity of these traits.

Alternatively, a founder effect may also explain the steeper change found at the Kerguelen Islands, as invasive species often suffer bottlenecks during the transport to the new location (Sax et al. 2007). Sax et al. 2007 emphasized that the resulting reduced genetic variance may increase the relative significance of genetic drift or gene flows (associated with repeated introduction events) on phenotype. Although this factor cannot be excluded, it may be of reduced importance here, as gene flows are largely precluded at the Kerguelen Islands. Indeed, compared to other invaded areas close to the native range and/or within commerce and tourism routes, the Kerguelen Islands are characterized by extreme geographic remoteness, low human visitation, and stringent biosecurity measures (Frenot et al. 2005; Lebouvier et al. 2011).

Contrasted thermal reaction norms support locally adapted life strategies and question future changes within the invaded area

The two populations produced contrasting thermal reaction norms for both wing size and shape. The overall positive slope of wing size with increasing temperature in individuals from the Kerguelen Islands is interesting as it contrasts with that observed in flies from Brittany and with the "Temperture-Size Rule" found in several other studies (Atkinson 1994; Angilletta et al. 2004; Atkinson et al. 2006; for studies in Drosophilidae species, see for instance Partridge et al. 1994; Debat et al. 2003; Kingsolver and Huey 2008). In addition, sources of shape variations that differ significantly among populations are likely to be adaptive, rather than regulated by morphogenetic constraints (Gilchrist et al. 2000). Reaction norms, which are the phenotypic expression of a genotype across an environmental range (Schlichting and Pigliucci 1998), are good candidates for testing local adaptations and measuring adaptive differences among populations as they are "all mixtures of adaptations and constraints" according to (Stearns 1989).

Shape variations induced by temperatures are common in insects wings (see for example work in *Drosophila* genus by Imasheva et al. 2000; Debat et al. 2003; 2008), yet remain difficult to correlate with functional constraints (Gilchrist et al. 2000; Debat et al. 2008). Information remains limited about shape patterns, aerodynamics and the associated adaptive significance for flight (Gilchrist et al. 2000). The thermal range tested in our study indicated limited changes in the anterior-proximal region of the wing. Thermal changes primarily altered the crossvein and posterior-distal region of the wing, which have been reported to be less constrained by functional flight needs (Dickinson et al. 1999; Gilchrist et al. 2000). Our laboratory results may consequently indicate life strategies that covary with wing components regarding temperature, but not necessarily with flight itself. Again, reproductive traits are viable candidates that are known to vary with temperature in insects (see for example Leather 1994; Geister et al. 2008). Remarkably, the wing components that changed with temperature in our study match the components that changed over time in wild females (*i.e.*, the posterior-distal region and crossvein).

At the Kerguelen Islands, the comparatively small wings increased in size under high growth temperatures, which may indicate (i) the counter-selection of large wings in the invaded area at low temperatures, but (ii) the possible return to large wings in the presence of warming. This assumption suggests that the parameter that inhibits large wing size at the Kerguelen Islands might decline in the future as a response to global warming. This observation indicates the possible future change of wing morphology within the invasive population of *C. vicina*, as this archipelago is among the most sensitive areas to global warming worldwide (Chown et al. 2008; Lebouvier et al. 2011). The comparative investigation of wing loadings over time in the invaded range would provide significant insights on the possible evolution of dispersal. Overall, the future allochronic change of the wing of *C. vicina* at the Kerguelen Islands is a challenging concern towards understanding its ongoing invasion, as global warming also facilitates earlier flight events, broadens seasonal activity periods, speeds up development rates, increases winter survival, strengthens human-assisted dispersal and extends invaded ranges (Robinet and Roques 2010).

Mapping allochronic change with thermal phenotypic plasticity

The non-linearity of the reaction norms for wing shape is interesting, as this phenomenon has been related to polyphenic species and reflects discontinuous patterns (see de Jong et al. 2010). As stressed by Moczek et al. (2002), polyphenism relies on threshold responses to produce discrete phenotypes, as opposed to graded phenotypes. These authors investigated polyphenism in *Onthophagus taurus* (Coleoptera, Scarabaeidae), and demonstrated different thresholds between native and invasive populations. Given that the flies naturally experience a narrow range of low temperatures in the invaded area (narrower than the range tested at the laboratory in this study, see Lebouvier et al. 2011), local responses specific to low temperatures may be questioned. This assumption may also contribute towards explaining the larger wing sizes observed under warm growth temperatures, which contrasted with the "Temperature-Size Rule". A supporting result for wing shape is the combination of PCO ordination and the cosines of angles between vectors. Indeed, it demonstrated that, at the Kerguelen Islands, the vectors depicting actual allochronic change in nature clustered with the shape vectors that corresponded to low temperature shifts. As shown for *Drosophila* wings by Debat et al. (2009), the temperature effect may alter the direction of available phenotypic variation in addition to changing selective pressures. Considering plasticity as a mechanism promoting different evolutionary solutions is controversial and has been long debated (see for instance de Jong 2005). However, several studies have discussed how phenotypic plasticity can alter the potential for evolution, as a primary parameter or in parallel with genetic variation (West-Eberhard 2003; Palmer 2004; Badyaev 2005; Braendle and Flatt 2006; Debat et al. 2009).

Perspectives

We combined allochronic and plastic explorations of the wing in an active flyer invading an area where flightlessness is a rule. This innovative biological system provides unique opportunities for tackling ecologically driven rapid evolution and delineating ecomorphology for flight. As a first step towards achieving these challenging tasks, we documented the morphological changes that actually occurred within this system, and discussed their potential outcomes. Further investigations and complementary data are now necessary to address the evolutionary significance for the changes described. As main priorities, molecular tools should be considered to test whether genetics underlie the distinct patterns reported. Furthermore, replicating the study at the intraspecific (more native populations of *C. vicina*) and interspecific (more invasive flyers at the Kerguelen Islands, albeit there are few) levels would allow drawing directional predictions for possible evolution of the wing at the Kerguelen Islands.

Huey et al. 2005 reported that evolutionary responses of alien species to selective pressures of the newly colonized environments can alter their spread and their interactions with the native biota, thus stressing the relevance of such studies in evolutionary biology, ecology and conservation. The needs for flight dispersal are likely to be lowered at the Kerguelen Islands, but the ability of *C. vicina* to reach food patches before competitors may be advantageous (Chevrier et al. 1997). However, deciphering the role of wing size and shape on dispersal ability in the naturalized population is a challenging topic, as we still lack detailed understanding of the functional constraints operating on wing morphology. Pursuing our study over the long-term would allow stating whether the changes are achieved or still processing. Further investigating the life-histories associated with the alterations of wing components emphasized here, and their variations among populations, would help understanding the role of phenotypic plasticity as a key for the success of species invading "naive" environments.

Acknowledgments This research was supported by the "Institut Polaire Francais" (IPEV 136), the CNRS (Zone Atelier de Recherches sur l’Environnement Antarctique et Subantarctique) and the "Agence Nationale de la Recherche" (ANR-07-VULN-004, EVINCE). This research is linked with the SCAR Evolution and Biodiversity in the Antarctic research programme. Mathieu Laparie is supported by a fellow from the MENRT (Université de Rennes 1, France), and Yann Cozic was funded by the programme EVINCE of the "Agence Nationale de la Recherche".

2.2. Aliens have to withstand their own ecological impacts (ARTICLE III)

Biological invasions are considered to be a major threat to biodiversity worldwide (Vitousek et al. 1996, 1997), so that the definition of invasive species has been frequently associated with their negative impacts on native biota (Falk-Petersen et al. 2006). Invasive predators are namely known to disrupt native prey communities, thereby progressively impairing the availability and quality of their own food resources (Kenis et al. 2008, Snyder & Evans 2006). Their ability to withstand their progressive impacts on local conditions is thus a challenging concern for understanding their durable success in disrupted ecosystems. Their negative feedbacks may interact over time with their own life history traits, phenotypic characters, or even genetic variance. This paradoxical durable success of invasive species in vulnerable ecosystems was studied in *M. soledadinus*, as the rapid impact of this predator on native biota has already been reported. We investigated morphological differentiation among populations of different residence times. The sites compared where similar except that the available prey communities are differentially altered depending on the residence time of *M. soledadinus*, from pristine communities to disrupted communities where some native species have been driven extinct. The example of *M. soledadinus* is particularly striking for these problematics, as the spread of this generalist predator has been monitored over the long term and local extinctions of native species recorded in real time. Moreover, the invasion of this species resulted from a single episode of introduction, ensuring that the possible morphological differentiation within the archipelago originates from the genotypes initially introduced at Port Couvreux in 1913.

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ORIGINAL PAPER

Variation of morphometric traits in populations of an invasive carabid predator (*Merizodus soledadinus*) within a sub-Antarctic island

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Abstract Invasive predators may change their own trophic conditions by progressively displacing or reducing diversity and abundance of native prey. As food quality and quantity are two main factors determining adult body size in arthropods, alteration of the available resources may thus affect predators' morphology. The flightless carabid beetle *Merizodus soledadinus* was accidentally introduced to Iles Kerguelen in a single site in 1913. Its successful spreading process has been monitored over the long term, providing an exceptional research opportunity with multiple snapshots of similar colonized sites mostly differing by the residence time of *M. soledadinus*. To test if *M. soledadinus*' morphology is correlated with its residence time in each habitat, we measured nine morphometric traits in five populations. We detected significant morphological differences: individuals from the first colonized site were the smallest, whereas individuals from the most recently colonized site were the largest. Our study also highlighted among-site variation in sexual dimorphism of the last abdominal sternite: its length differed between sites for females, but not for males. We discuss this diminution of *M. soledadinus*' size in the light of both a priori (development under diet restriction, survival) and a posteriori (intrapopulation competition, cannibalism) effects on growth and development.

Keywords Trophic resources · Phenotypic plasticity · Morphometrics · Body size · Biological invasion · Insect · Predator · Gender · Sub-Antarctic islands

Introduction

Several parameters, from physiological processes to environmental pressures, play a role in determining the body size and related morphological parameters in insects. Among a wide range of factors, ontogenesis, biomechanical constraints, sexual selection, fecundity, size-specific predation, resource quality and availability, overcrowding, competition and temperature have often been reported as the most prominent ones (Angilletta and Dunham 2003; Berven and Gill 1983; Juliano 1986; Wheeler 1996). Most of these factors may vary from one habitat to another and geographic variation in body size has thus been studied extensively (Boggs and Freeman 2005; Chown and Klok 2003; Schmidt-Nielsen 1984).

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At a large geographic scale, clinal variation of morphological parameters within species from different taxa has been found (Blanckenhorn and Demont 2004; Hallas et al. 2002). The nature of such variation has been addressed frequently along altitudinal and/or latitudinal climatic gradients (Arthur et al. 2008; Blanckenhorn et al. 2006). However, the mechanisms driving a differential expression of the genotype over a large range of thermal environments are not fully understood (Angilletta and Dunham 2003; Cabanita and Atkinson 2006). Fewer studies have examined divergence in the expression of morphological traits at local geographical scales, where trophic resources and trophic competition appear as prime determinants of adult size and morphology. When nutritional resources are limiting, metabolic trade-offs constrain the allocation of energy inputs to growth, somatic maintenance and reproduction [as shown for example in *Allomyrina dichotoma* (Coleoptera, Scarabaeidae) by Moczek (1998), and in *Onthophagus taurus* (Coleoptera, Scarabaeidae) by Karino et al. (2004)]. In holometabolous insects, restriction in the quality and quantity of the larval diet may result in altered adult morphology and fitness (Boggs and Freeman 2005), with imagos exhibiting specific allometric relationships among various body parts such as wings, flight muscles, ovaries and head.

Within insect species, the size of each organ, appendage or body region bears a specific relationship to overall body size (Shingleton et al. 2007). Positive correlations have been found between wings and body size (Stern and Emlen 1999), fore-femur length and body size (Stern et al. 1996), and body size and morphological traits associated with feeding (mandibles, head) (Thompson 1992). Besides these correlations, head width or mandible length can be related to an ability to consume larger food items (Pearson and Stemberger 1980). Such a positive relationship between feeding morphology and body size might also differ among populations because of distinct resource availability or foraging strategies. This is particularly significant in many predatory arthropods, often food-limited in natural situations (Bommarco 1998; Pearson and Knisley 1985), and for which food intake provides a major part of the resources used for reproduction (Juliano 1986; Sota 1985; Wise 1979).

Invasive predators appear even more affected by availability of resources. They often change their own trophic conditions by displacing or reducing diversity and abundance of native prey (Kenis et al. 2008; Snyder and Evans 2006), as found in the ground beetle *Merizodus soledadinus* Guérin-Méneville 1832 (Coleoptera, Carabidae) (Chevrier 1996). This insect was introduced from the Falkland Islands to a single site (Port Couvreux) on Iles Kerguelen in 1913 (Jeannel 1940), where it has no efficient competitor [the only native predator species on Iles Kerguelen are one staphylinid, *Antarctophytosus atriceps* (Coleoptera, Staphylinidae) and two spiders, *Myro kerguelensis* (Araneae, Desidae) and *Neomaso antarcticus* (Araneae, Linyphiidae)]. During the past century, this flightless ground beetle has spread over the eastern part of the archipelago and colonized several habitats far from Port Couvreux. This spread has resulted in the formation of geographically distant populations characterized by distinct residence times in each habitat. Because the temporal evolution of the distribution of this alien predator was monitored, we have a set of snapshots ranging from formerly to recently colonized habitats. In addition, this insect can rapidly become a dominant species once established in a new site (Chevrier et al. 1997). The quality and quantity of available resources may be quickly altered and differ among colonized habitats according to the residence time of *M. soledadinus*, impacting individuals' development and morphometry. Also, most animals exhibit a sexual dimorphism mainly related to different exploitation of food resources by females for reserve storage (fat, proteins) for egg maturation, and to reduced longevity in males (Atchley 1971; Butler 1986; Fairbairn 1997). *M. soledadinus* exhibits a sexual size-related dimorphism (Chevrier 1996), hence its growth ability may be differentially affected in males and females by short-term variation in food abundance.

Using a morphometric analysis, we asked if long-term colonization process on Iles Kerguelen induced differences between *M. soledadinus* individuals. To address this question, we measured nine quantitative parameters in adults sampled in five distinct sites colonized at distinct periods. We hypothesized that (i) individuals of *M. soledadinus* are characterized by reduced sizes and morphological traits in habitats colonized early because this predator altered the amount of available trophic resources, (ii) food limitation would lead to divergence in food-gathering characters and (iii) the selection pressure resulting from the availability of food resources affect males and females differentially.

Materials and methods

Collection sites and insect sampling

Wild specimens of *M. soledadinus* were sampled in December 2006 in five distinct sites (Fig. 1): (1) Port Couvreux (49°16′35.3″S, 69°41′5.1″E) where the species was first introduced to Iles Kerguelen in 1913 (Jeannel 1940), (2) Port Elizabeth (49°12′58.2″S, 69°51′57.6″E) (first individuals observed *ca.* 1970—Chevrier 1996), (3) Port Jeanne d'Arc (49°33′0.3″S, 69°48′18.2″E) (first observation in 1991—Chevrier 1996), (4) Ile Guillou (49°28′17.6″S, 69°48′23.4″E) (first observation in 1995—Lebouvier, unpublished data) and (5) Pointe Guite (49°25′22.5″S, 70°16′53.6″E) (first observation *ca.* 2003—Lebouvier, unpublished data). Literature and long-term monitoring schemes conducted on Iles Kerguelen since 1974 ensure the accuracy of these arrival dates, except for Port Elizabeth, where the estimated arrival date falls between 1939 and 1970. Based on these data, we can determine the residence time of *M. soledadinus* in each site (Fig. 2). For the five sites, each batch of measured individuals will be considered as a "population."

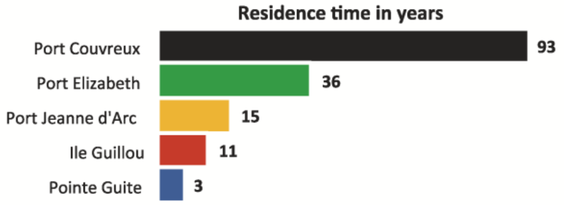


Fig. 2 Residence time in each habitat when the five populations of *Merizodus soledadinus* were sampled (2006)

Imagos of *M. soledadinus* were all hand-collected from December 1st to 15th 2006 in coastal areas under tide drift lines, so there were strong similarities among sampled habitats. Because of the reduced geographical distance between the sites (less than 50 km) and the absence of major topographic barriers impacting temperature or rainfall, all collected individuals experienced similar meteorological conditions whatever the collection site (meteorological data—available for Port-aux-Français since 1950 and for Ile Guillou since 1997—confirmed this assumption). To ensure a

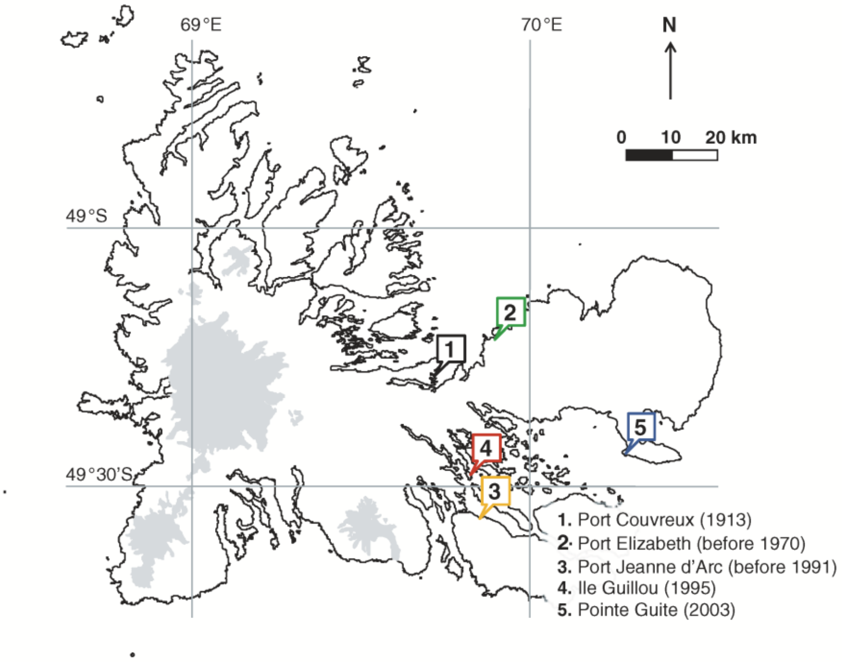


Fig. 1 Localization of the five distinct sampled sites and their colonization dates by the ground beetle, *Merizodus soledadinus*

sufficient number of insects for each sex to conduct the study and the subsequent statistical analysis, 100 imagos were randomly caught in each site and were immediately stored in vials filled with 70% ethanol before being measured in the lab.

Traits measured

We measured nine morphometric traits (Fig. 3) in 30 males and 30 females for each one of the five sampled sites (except for Port Jeanne d'Arc, where only 26 males were in a preservation state that allowed reliable measurements of all the traits).

Pictures of each measured parameter were taken for each specimen with a video camera (720 × 576 pixels) connected to a binocular microscope. Measurements were done by vectorial layouts with *JMicroVision 1.2.5* (Geneva, Switzerland). Theoretical precision was 8.8 µm at ×6.4 magnification (used for TOT and ELYT, see Fig. 3 for a detailed description of the traits measured), 3.3 µm at ×16 (used for INTOC, $\mathrm{PRONO_L}$, $\mathrm{PRONO_W}$, $\mathrm{FEMU_1}$, $\mathrm{FEMU_3}$ and STER) and 0.9 µm at ×40 (used for PALP). Each appendage was placed perpendicularly to the video camera axis to limit parallax problems. Right legs of the first and the third pairs of legs were detached for measurement of $\mathrm{FEMU_1}$ and $\mathrm{FEMU_3}$.

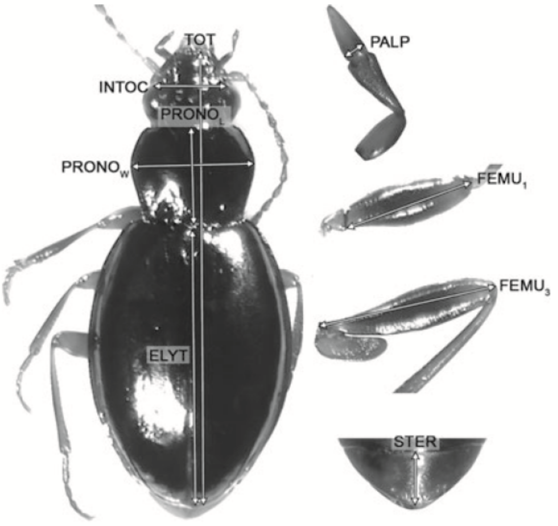


Fig. 3 Morphological traits measured in adults of *Merizodus soledadinus* (male shown). *TOT* Individual total length from the labrum to the end of the right elytra, *ELYT* Length of the right elytra, *INTOC* Fore interocular width, $\mathit{PRONO_L}$ Length of the pronotum, $\mathit{PRONO_W}$ Width of the pronotum, *STER* Length of the last abdominal sternite, $\mathit{FEMU_1}$ Metafemur length of the right foreleg, $\mathit{FEMU_3}$ Metafemur length of the right hind leg, *PALP* Width of the last article of the right maxillary palp

After the measurements, each individual (with previously detached legs) was dried for 5 days at 60°C and then weighed with a Mettler H20 microbalance ($d = 10$ µg). Mass is strongly related to size in ground beetles (Hodar 1996; Jarosik 1989) and this mass measurement (MASS) allowed us to compare individuals between sites, but it does not strictly correspond to dry mass because of the solubilization of several compounds in ethanol (den Nijs et al. 1996). However, den Nijs et al. (1996) showed a significant correlation between alcohol mass and dry mass in adults of *Pterostichus cupreus* (Coleoptera, Carabidae).

Statistical analysis

A bias was identified in measurements of the total length (TOT) of individuals: because of their articulations, the distance between tagms (head, thorax and abdomen) differed greatly among individuals. The bias was confirmed by the low repeatability of this measure on each specimen. In addition, ethanol storage may modify insect abdomen size by swelling or distension (Gruner 2003). Hence, TOT was not used for the multivariate analyses. High variation was also found for dry mass (MASS), but this measure was reliable and reflects individual variability.

Adequate normality of the distribution of residuals was checked for each variable, each sex and each site by Q–Q plots and Shapiro–Wilk tests; Levene tests were applied to test homoscedasticity of the variables. A two-way MANOVA was then performed to test the significance of the factors (sex and site) with respect to potential correlations among variables. This multivariate analysis was followed by a Factorial Discriminant Analysis (FDA) for each sex to determine how the individuals from the five sites were structured according to the measured variables. Between- and Within-Group analyses were also used for each sex to determine the proportion of inertia explained by the differences among sites (inter-site variability) on the one hand, and by heterogeneity within sites (intra-site variability) on the other. Separation and neighbourhood of the sites observed on both discriminant planes were fully confirmed using Hierarchical Ascendant Classifications (HAC) on measured variables, with a priori input of five groups (data not shown).

A univariate procedure was performed to explore further the sex × site interaction. Interaction plots showed only an interaction for the STER parameter. A two-way ANOVA was therefore performed for STER to test (i) differences among sites for STER in each sex (site effect), (ii) sexual dimorphism for STER in each site (sex effect), and (iii) variation among sites in the expression of the sexual dimorphism for STER (sex × site interaction). Pairwise comparisons were performed by the Tukey post hoc procedure.

All statistical tests were done with R^{TM} 2.7.0 with an α threshold = 0.05. Multivariate analyses were run with ADE4 plugin for R^{TM} 2.7.0 (Thioulouse and Dray 2007).

Results

Sexual dimorphism and morphological differences among sites

Mean trait values for each sex and each site are shown in Table 1 with the MANOVA results. A strong and significant sexual dimorphism appeared, as well as significant morphometric differences among sites. Interaction plots showed the highest sex × site effect for STER (Fig. 4). This suggests that sexual size dimorphism expression was more altered by the site effect for the last abdominal sternite than for the eight other parameters and for the mass. As shown in Fig. 4, STER sexual dimorphism was highest in Port Elizabeth—with females showing the highest values—whereas it was similar in the other sites.

STER sexual dimorphism was further examined using the Tukey post hoc procedure (Fig. 5). Sexual dimorphism for STER was significant only in Port Couvreux, Port Elizabeth and Ile Guillou. Sexual dimorphism was highest in Port Elizabeth and lowest in Port Jeanne d'Arc. Moreover, STER did not differ significantly among males, whereas differences appeared among females (Fig. 5).

Variation of morphological traits in males

The MANOVA confirmed the significant differences among populations. FDA was performed to determine which traits act to separate the populations. In males,

Table 1 Values (mean ± SD) of the traits measured in males and females sampled in the five sites on Iles Kerguelen

	1. Port Couvreux		2. Port Elizabeth		3. Port Jeanne d'Arc		4. Ile Guillou		5. Pointe Guite		MANOVA		
	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	Factor	$F_{d.d.l.}$	*P*
TOT	5677 ± 320	5977 ± 371	5568 ± 229	5908 ± 248	5685 ± 181	5883 ± 227	5550 ± 215	5828 ± 280	5832 ± 219	5907 ± 283	Sex	35_9	***
ELYT	3168 ± 111	3336 ± 141	3202 ± 114	3411 ± 117	3236 ± 102	3371 ± 139	3227 ± 106	3397 ± 157	3288 ± 123	3423 ± 118	Site	8_{36}	***
INTOC	859 ± 36	872 ± 37	879 ± 34	912 ± 34	895 ± 34	907 ± 24	893 ± 32	919 ± 35	897 ± 35	906 ± 32	Sex × site	2_{36}	***
$PRONO_L$	1103 ± 45	1137 ± 57	1111 ± 53	1175 ± 42	1117 ± 42	1161 ± 51	1121 ± 48	1176 ± 60	1139 ± 45	1158 ± 50			
$PRONO_W$	1453 ± 55	1479 ± 85	1485 ± 58	1538 ± 68	1473 ± 78	1546 ± 69	1517 ± 59	1562 ± 76	1488 ± 78	1545 ± 54			
STER	540 ± 29	582 ± 36	522 ± 36	589 ± 36	517 ± 31	523 ± 37	520 ± 37	551 ± 49	546 ± 40	572 ± 36			
$FEMU_1$	1068 ± 40	1073 ± 46	1063 ± 52	1092 ± 41	1046 ± 52	1080 ± 49	1062 ± 40	1085 ± 56	1094 ± 50	1100 ± 39			
$FEMU_3$	1443 ± 54	1458 ± 67	1442 ± 68	1478 ± 53	1424 ± 60	1450 ± 62	1435 ± 66	1462 ± 79	1488 ± 58	1487 ± 64			
PALP	75 ± 5	78 ± 4	80 ± 5	81 ± 3	80 ± 4	83 ± 4	80 ± 3	83 ± 3	80 ± 4	82 ± 3			
MASS	2062 ± 381	2547 ± 463	2279 ± 280	2572 ± 454	2040 ± 258	2344 ± 342	2321 ± 353	2783 ± 558	2785 ± 397	3032 ± 574			

TOT Individual total length from the labrum to the end of the right elytra, *ELYT* Length of the right elytra, *INTOC* Fore interocular width, $PRONO_L$ Length of the pronotum, $PRONO_W$ Width of the pronotum, *STER* Length of the last abdominal sternite, $FEMU_1$ Metafemur length of the right foreleg, $FEMU_3$ Metafemur length of the right hind leg, *PALP* Width of the last article of the right maxillary palp, *MASS* Alcohol-dry mass of body + detached legs

Biometrics are expressed in μm and mass in μg. 30 males and 30 females were measured for each site, except in Port Jeanne d'Arc ($N_{males} = 26$)

*** $P \leq 0.001$

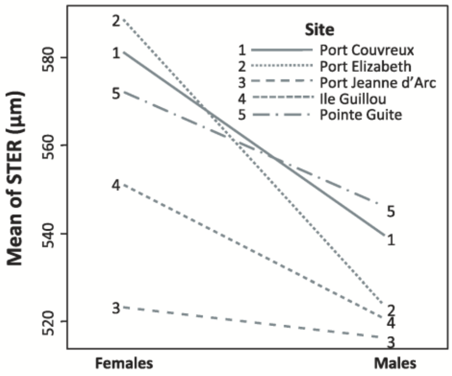


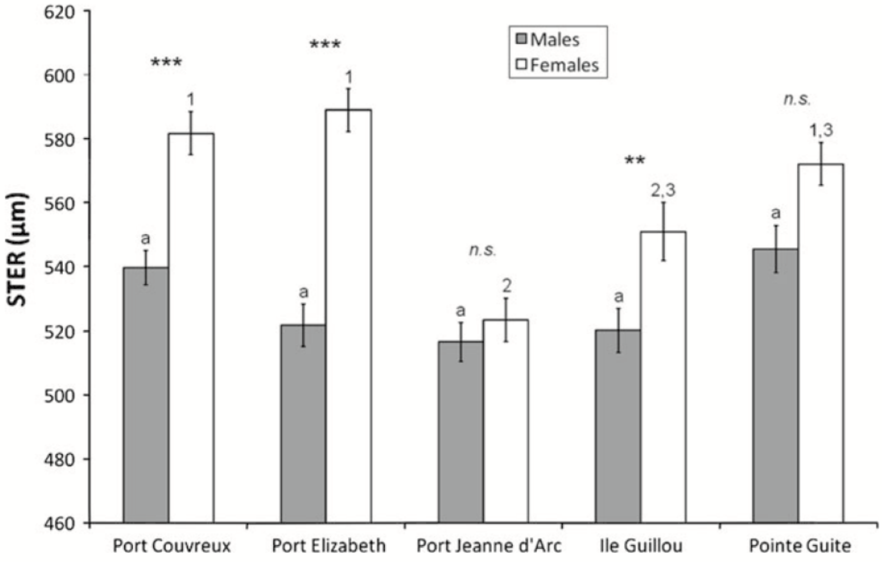
Fig. 4 Interaction plot (sex × site) for STER (length of the last abdominal sternite). Sex effect appeared higher in Port Elizabeth than in the four other sites

the first factorial plane (F1 × F2) accounted for 82.2% of the total inertia (Fig. 6). Groups were significantly separated by the first and second axes (Wilks $\lambda \approx 0$, Bartlet χ^2, $P < 0.001$ for F1 and F2). The correlation circle and structure matrix (data not shown) indicated that F1 is a general body size axis (contributions from ELYT, FEMU_1, FEMU_3, PRONO_L and MASS), with negligible contributions from STER, PALP, INTOC and PRONO_W. The second axis (F2) mainly corresponded to a contrast between STER and PALP plus INTOC.

Individuals coming from the habitats with the longest (Port Couvreux) and shortest (Point Guite) residence times were opposed on the first discriminant axis (Fig. 6): the smallest individuals were from Port Couvreux (1) and the largest from Pointe Guite (5). Individuals from populations 2, 3 and 4 had intermediate positions and were poorly discriminated on F1 axis. The F2 axis sorted populations as follows: 1; 5; 2; 4 and 3. Individuals from Port Couvreux (1) were well discriminated owing to the particularly low values of their PALP (see Table 1).

Per site discriminations were not completely achieved, and overlapping between populations appeared. In Port Couvreux (1) and Pointe Guite (5), males were the best assigned to their group (respectively 78 and 70%). Individuals from Port Jeanne d'Arc (3) and Ile Guillou (4) were well separated too (respectively 65 and 63%), but most of the males from Port Elizabeth (2) were misclassified, with only 30% well assigned. This finding may be linked to the results of the Between- and Within-Group Analyses, which showed that inter-site differences accounted for only 15% of the total inertia, while intra-site heterogeneity accounted for most of the inertia (85%). These results point to the significant effect of the different sites as well as important intra-site variability caused by high inter-individual heterogeneity. The relative standard deviation (RSD) was used to quantify the variability between males within each population. For all variables, the RSD reached 6.0% in Port Couvreux, 5.6% in Port Elizabeth,

Fig. 5 Mean STER (SE) in males and females sampled in the five distinct sites. Sites are sorted by colonization dates, from Port Couvreux (1913) to Pointe Guite (*ca.* 2003). Between sexes: *** $P \le 0.001$, ** $P \le 0.005$; *n.s.* not significant. Between sites: *different letters* (males) and *numbers* (females) indicate significant differences between samples



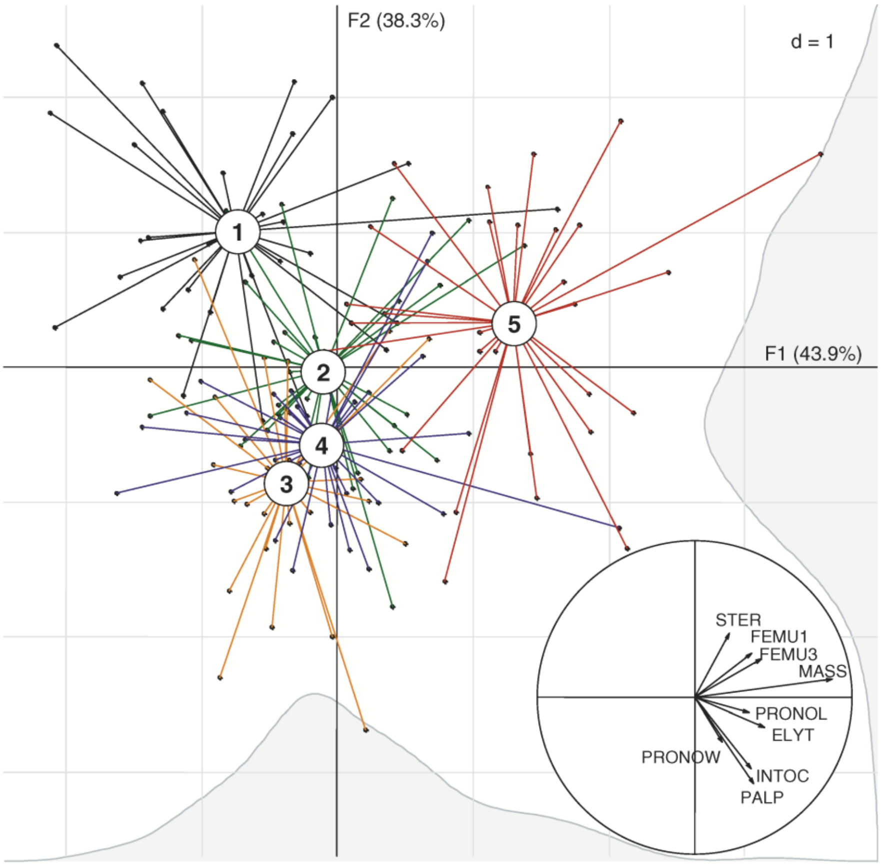


Fig. 6 Factorial Discriminant Analysis: projection of variables (*correlation circle*) and males of each population onto the first factorial plane. *Lines* link individuals to the centre of gravity of their group. *Gray curves* illustrate density distribution of projected individuals on each axis. The amounts of inertia explained by axes F1 and F2 are expressed as percentages. *1* Port Couvreux, *2* Port Elizabeth, *3* Port Jeanne d'Arc, *4* Ile Guillou, *5* Pointe Guite

5.4% in Port Jeanne d'Arc, 5.4% in Ile Guillou, and 5.7% in Pointe Guite.

Variation of morphological traits in females

Overall, conclusions were similar for females except that the first factorial plane was rotated. The sign relationship of the F1 and F2 axes was inverted and did not account for the same amount of inertia as in males (Fig. 7). This first factorial plane accounted for 77.6% of the total inertia, with significant discrimination on the two-first axes (Wilks $\lambda \approx 0$, Bartlet χ^2, $P < 0.001$ for F1 and F2). The F1 axis mainly discriminated populations by the contrast between STER and PALP plus INTOC (as for the males' F2 axis). F2 was an increasing general body size axis, mainly constructed by MASS, ELYT, $FEMU_1$ and $FEMU_3$. Port Couvreux (1) and Port Jeanne d'Arc (3) individuals were opposed on the F1 axis, and Pointe Guite (5) individuals were well separated on the F2 axis.

Misclassifications also appeared in females. In Port Couvreux (1), Port Jeanne d'Arc (3) and Pointe Guite (5), females were the best assigned to their groups (respectively 73, 70 and 67%), whereas only 50 and 40% of the individuals from Port Elizabeth (2) and Ile Guillou (4) were well assigned, respectively. As in males, Between- and Within-Group Analyses indicated high intra-site variability: inter-site and intra-site differences accounted for 13.7 and 86.3% of the total inertia, respectively. For the eight reliable measurements, the RSD were 6.4% in Port Couvreux, 5.6% in Port Elizabeth, 5.6% in Port Jeanne d'Arc, 6.9% in Ile Guillou, and 5.7% in Pointe Guite.

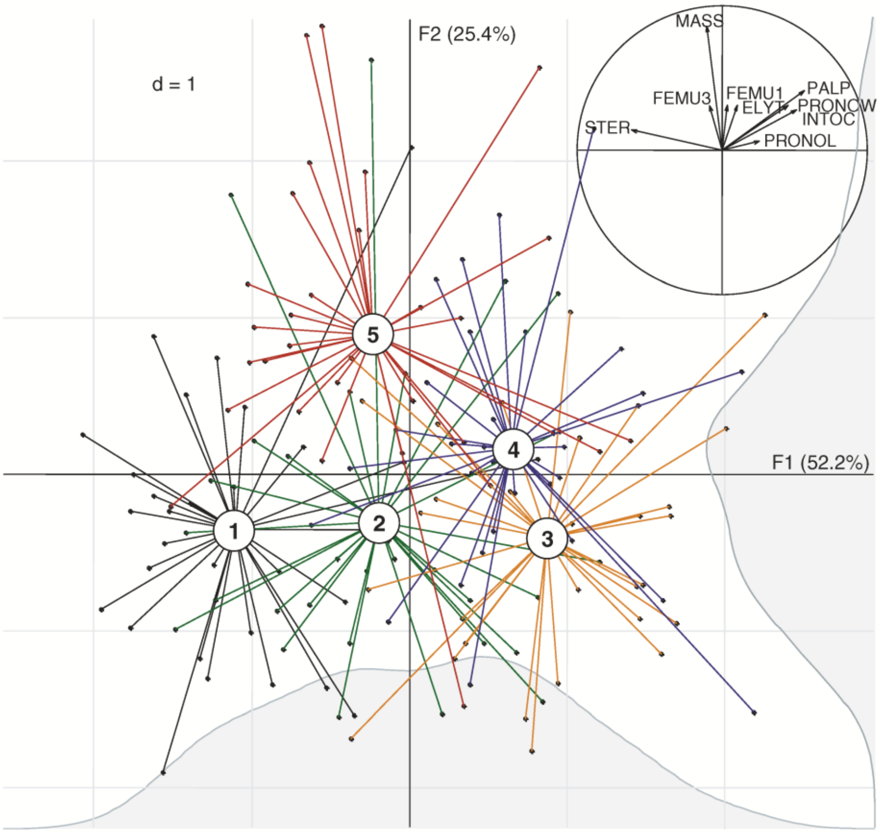


Fig. 7 Factorial Discriminant Analysis: projection of variables (*correlation circle*) and females of each population onto the first factorial plane. *Lines* link individuals to the centre of gravity of their group. *Gray curves* illustrate density distribution of projected individuals on each axis. The amounts of inertia explained by axes F1 and F2 are expressed as percentages. *1* Port Couvreux, *2* Port Elizabeth, *3* Port Jeanne d'Arc, *4* Ile Guillou, *5* Pointe Guite

Discussion

The importance of examining variation of morphological traits was recently re-emphasised because these traits (i) are used extensively for taxonomy, (ii) are partially under genetic control, (iii) are the target of selection, and (iv) reflect intraspecific clinal divergence (Garnier et al. 2005). Moreover, variation in morphology can exhibit clear patterns of differentiation that molecular markers may not detect (Nice and Shapiro 1999).

Imagos of *M. soledadinus* were collected at the same times on the Péninsule Courbet (Eastern part of Iles Kerguelen) in similar microhabitats (under tide drift lines and stones along the seashore). Climatic conditions differ greatly between the western and eastern part of Iles Kerguelen. The western region is mountainous and experiences Foehn winds. The eastern region, and more particularly the Péninsule Courbet, is composed of large coastal plains, where climatic conditions at sea level are similar. This assumption is supported by our meteorological data collected on Ile Guillou and at Port-aux-Français at a depth of five centimetres below ground level. The size-trait divergence patterns observed among the five distinct sites on Iles Kerguelen can thus be related to the residence time of *M. soledadinus* in each location, i.e. to the distinct abundance and availability of the trophic resources as a result of predation pressure (Chevrier 1996; Chevrier et al. 1997; Laparie, Lebouvier and Renault, unpublished data), rather than to differential abiotic conditions in the microhabitats.

Variation in body size, either at the individual level or in the frequency distribution of individuals' body sizes within a population, may indicate different types of environmental stress (McGeoch 1998). Nutrition is one of the best studied factors that affect morphometry and that can vary among habitats (Shingleton et al. 2007). In our study, in both sexes, morphometry of individuals from Port Couvreux (highest residence time) was always opposed to that one of individuals from Pointe Guite (shortest residence time), both being extremes in terms of general body size. As assumed by the "decreasing body size hypothesis" (Blake et al. 1994; Gray 1989; Szyszko 1983), highly disturbed habitats support smaller ground beetles more than less disturbed areas do (Magura et al. 2006). This probably results from their lowest energetic requirements for growth and their shortest durations of development (Peters 1983). Given the low arthropod diversity on Iles Kerguelen, the two native wingless flies *Anatalanta aptera* (Diptera, Sphaeroceridae) and *Calycopteryx moseleyi* (Diptera, Micropezidae) represent two of the most profitable prey for *M. soledadinus* in terms of energy (Vernon 1986) and handling time (including spotting, capture, eating and digesting—see Krebs and Davies 1993). Over the last 25 years, no *A. aptera* or *C. moseleyi* were observed at Port Couvreux and very few were observed near Port Elizabeth although they used to be abundant in both sites (Chevrier 1996; Lebouvier, unpublished data). These species disappeared from Port aux Français as soon as *M. soledadinus* arrived there (1995) and could still be observed some few kilometers away at Pointe Guite in 2006, where some adults of *M. soledadinus* were also observed for the first time. Populations of *M. soledadinus* were found to have a strong impact on invertebrate diversity and abundance (Chevrier 1996; Lalouette, Lebouvier and Renault, unpublished data). A potential predation switch to microinvertebrates such as springtails when the more profitable macroinvertebrate prey like the native flies become scarcer could explain the body size decrease. In addition, a morphometric differenciation also appeared in populations characterized by recent and close residence times, supporting earlier conclusions of Chevrier et al. (1997) that *M. soledadinus* quickly becomes the most abundant species in invaded habitats.

Besides the overall body size, shape and size of trophic appendages were found to vary between individuals supplied with distinct diets (Thompson 1992; Thompson 2001). Divergence in mouthparts may result in an asymmetry of the prey consumed (Pearson and Stemberger 1980). The thinnest maxillary palps and smallest heads were found in individuals from Port Couvreux, but there were no significant differences among individuals from other sites for those variables. In light of the significant morphological differences among individuals from Port Elisabeth, Port Jeanne d'Arc, Ile Guillou and Pointe Guite, biometric evolution of both head and maxillary palps cannot be coupled to changes in size of the other morphological variables. The residence time in each habitat could be an influential variable in the morphology of *M. soledadinus*. We hypothesize that alteration of available resources, and the resulting prey-switching, act on the general morphology of the individuals and determine the evolution of the mouthparts parts' size.

Body size of *M. soledadinus* can also be altered by intrapopulation competition. Lenski (1982) showed that body size was reduced when density and competition increased in *Carabus limbatus* (Coleoptera, Carabidae). During the survey of the geographic distribution and abundance of *M. soledadinus* in 2005–2006, we quantified population densities on Iles Kerguelen. We defined 4 abundance levels from 1 (no individual found after a 10 min search by one person to 4 (very high densities, more than 250 individuals found in 10 min by one person). The five locations sampled in the present study were characterized by the highest abundance level (level 4, very high densities), thus suggesting intrapopulation competition. Despite significant discrimination of the five sampled populations, high variability occurred within each population. In the parasitic beetle *Brachinus lateralis* (Coleoptera, Carabidae), host size is of prime importance to individual size, but the limited opportunity for host choice may maintain size variation despite evidence of natural selection pressure on size (Juliano 1985). Intrapopulation competition may maintain size heterogeneity in *M. soledadinus* owing to different food intakes between individuals, regardless of the overall habitat resources. Developmental instability, i.e. intra-individual stochastic variation and phenotypic noise during growth (Nijhout and Davidowitz 2003; Dongen 2006), could also maintain variability within populations regardless of the factors studied here. In addition, the life cycle of *M. soledadinus* lasts about 1 year and its lifespan exceeds 1 year (Jeannel 1940; Ernsting 1993), suggesting that we should have more than a single cohort within each population. Variation in adult body size may also result from different thermal conditions larval instars endured during their development (Angilletta 2009). Although there are no hints suggesting different population structure in different sites, we can therefore not rule out a possible effect on body size.

Cannibalism may also impact body size when both quality and quantity of food are reduced. This phenomenon was described as prevalent when food resources are limiting because it reduces competition for these resources (Dong and Polis 1992; Currie et al. 1996). More cannibalistic interactions may occur in the first habitats colonized by *M. soledadinus*. When breeding *M. soledadinus* populations under controlled conditions, we observed predation only of imagos on larvae (no predation by larvae of one another). As smaller individuals develop faster than bigger ones under similar conditions (as shown by Blanckenhorn (1998) in *Scatophaga stercoraria* (Diptera, Scatophagidae)), small individuals of *M. soledadinus* may be less penalized by reaching the adult stage more quickly. Indeed, Chown and Nicholson (2004) reported that traits promoting large body size such as extended development time or increased growth rate (which depends on increased feeding rate) increase the risk of predation. In our study, the residence time could promote a body size diminution and a faster development owing to resource limitation and higher intrapopulation competition in the most altered sites. However, we also must keep in mind that larvae have a different space-approaching behaviour than adults, i.e. they bury themselves and are thus found in the soil, whereas adults are usually found at ground level.

As was previously demonstrated by Chevrier (1996), we found a significant sexual dimorphism in adults of *M. soledadinus*, with females larger than males. This result is consistent with the literature, since several insect species have already been characterized by marked sexual dimorphism in either mass, size, shape or even physiological capabilities (Day et al. 1994; Fairbairn 1997; Renault et al. 2003; Svensson 1977). Because of their different roles in reproduction, males and females are often under selection that favours their divergent morphological appearance (Badyaev 2002). Energetic allocation contributes greatly to explaining sexual size dimorphism, with females having a higher energy cost associated with gamete production (Nylin and Gotthard 1998; Tammaru et al. 2002). On the other hand, male size may affect their dispersal and be important in sexual selection and competition for females (Nylin and Gotthard 1998).

The morphological traits covarying with reproductive and dispersal functions are of particular interest with respect to the invasive success of a species. They represent the level of developmental and morphological plasticity of the species to the environmental and ecological characteristics of the newly colonized habitat (Chown et al. 2007; Rosecchi et al. 2001). Reproductive traits and more particularly copulatory organs often exhibit reduced variation relative to body size within species of arthropods (Eberhard 1998). We found that STER variation was not paired with body size variation. STER values in males were similar in all sites regardless of body size variation. In females, STER values did not differ between the smallest individuals (Port Couvreux and Port Elizabeth) and the biggest ones (Pointe Guite). Energetic allocation during diet restriction thus involves differential developmental plasticity for STER. The target of sexual selection could be reproduction-related traits rather than body size, as was found previously by Preziosi and Fairbairn (1996). Because insects may have greater success with larger genitalia and not because they are bigger *per se*, the general body size-independent variation of STER is ecologically relevant. In addition, a stronger influence of reproduction on morphological traits in females than in males can be hypothesized. In Port Elizabeth, the sternites are short in males and long in females compared to the sternites of individuals from other populations. This may result from a greater difference in energetic allocation to reproduction between females and males in this population. Port Elizabeth could be a transitional stage in which reaction norms optimizing fecundity are expressed earlier in females. The relationship between STER and fecundity should be investigated further in *M. soledadinus* by measuring the number and size of ovarioles and eggs.

An integrative conjecture can be hypothesized to explain the relationship between morphometry and the invasion process in populations of *M. soledadinus*. In both sexes, differentiation occurred through body size (MASS, ELYT, FEMU$_1$, FEMU$_3$, PRONO$_L$) and reproduction (STER) *versus* feeding strategy (PALP, INTOC). Life histories promoting increased body size might be prominent when a species colonizes new sites, as was found in individuals from Pointe Guite. Larger individuals may be characterized by higher dispersal ability (Thiele 1977) and may therefore be more efficient than smaller ones in colonizing and establishing populations in new sites. Subsequently, reproduction strategies and adjustments of the feeding behavior as a consequence of the available prey in the microhabitat may be important for the settlement of the populations, as suggested by the characteristics of Port Couvreux individuals.

Blanckenhorn (2000) highlighted the need for experimental and comprehensive studies that address the fitness costs at the ecological level of being larger. Selection of large body size occurs in most organisms and empirical evidence is needed to determine if sporadic selection in time and space suffices to counterbalance this major evolutionary force (Blanckenhorn 2000). In the present study, we demonstrated morphological differentiations at a local scale among several populations differing from both geographical and chronological standpoints. We suggest that the observed size diminution of *M. soledadinus* may reflect both a priori (development under diet restriction, survival) and a posteriori (intrapopulation competition, cannibalism) effects on development and selection. Our study indicates that being smaller might be advantageous in habitats where optimal trophic resources are altered by *M. soledadinus*, a view that supports some of the mechanisms proposed by Blanckenhorn (2000).

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2.3. Size-selection through invasion process: is dispersal better on the front? (ARTICLE IV)

Species invasions are natural experiments that offer unique opportunities to observe ecological and evolutionary processes in real time (Sax et al. 2007). In particular, the evolution of dispersal during the spread stage of the invasion process is a crucial concern that encompasses both evolutionary questions and management policies, as it determines the range, extent, and consequences of invasive species (Phillips et al. 2006). Dispersal ability must be considered as a dynamic attribute of expanding populations, especially along the invasion front where it may be promoted (see theoretical models and validation with data on invasive toads in Phillips et al. 2008). The morphological differentiation among invasive populations of *M. soledadinus* previously found at the Kerguelen Islands (**ARTICLE III**) may result from such evolutionary changes. The relevance of this hypothesis was statistically examined in the following note, using the morphological data presented in **ARTICLE III**.

Laparie, Renault *et al*. *Submitted to Biological Invasions* Article IV

Is dispersal promoted during range expansion of invasive species? A synchronic approach using morphometrics in a ground beetle invading sub-Antarctic islands

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Abstract

As an invasive species is naturalized in a novel environment, its subsequent spread may promote dispersal ability by direct or indirect selective effects, thereby accelerating its range expansion and increasing the extent of its impacts. Morphology correlates with dispersal in numerous taxa, and may represent a relevant integration of temporal or geographical changes in dispersal power. Using the synchronic morphological data from Laparie et al. (2010), we examined the quantitative relationship between residence time and a multivariate combination of morphological parameters in *Merizodus soledadinus*, a ground beetle accidentally introduced into a single point of the Kerguelen Islands in 1913. We describe a consistent fingerprint of dispersal promotion regarding residence time, from the founder population to successive child populations. Indeed, the morphological differences among populations quantitatively matched their differences in residence time, depicting an increasing size gradient in dispersal-related traits along the colonization history of the species. Our results offer a fertile ground for investigating spatial selection and promotion of dispersers on invasion front margins, as they highlight the dynamic fashion of dispersal ability during biological invasions.

Keywords

Biological invasion, body size evolution, insect, Kerguelen Islands, *Merizodus soledadinus*, movement.

Introduction

Species invasions provide interesting insights on ecological and evolutionary processes (Davis 2009; Sax et al. 2007). As an invasive species is naturalized in new areas, its subsequent spread is likely to promote dispersal ability by direct or indirect selective effects (Holt 2003). Indeed, better dispersers are more likely to colonize new habitats and found new populations. The evolution of dispersal is a crucial concern in managing invasive species, as it may dramatically accelerate their range expansion and the extent of their impacts (Phillips et al. 2006), which are their most problematic features.

Since the 20th century, the multiplication of range shifts (Parmesan 2006) and biological invasions (see Davis 2009 and references therein) provided unique opportunities for monitoring the evolution of dispersal. Numerous species increased the variety of habitat types they can establish in (Thomas et al. 2001), or their movement ability and energy allocation to dispersal, often at the cost of reproduction (Roff 1986). Such changes range from flight muscles mass (Zera and Denno 1997) or frequency of long-winged forms in populations of insects (Roff 1986) to the proportion of dispersive seeds in plants (Cheptou et al. 2008). Trade-offs between dispersal traits and other traits upon dispersal evolution were namely reported in Carabids (Matalin 1994).

The sub-Antarctic Islands have been described as "the sentinels of the south", as they represent natural laboratories for ecologists and evolutionary biologists (Bergstrom and Chown 1999). Their extreme sensitivity to global changes (climate changes, species introductions), their paucispecific communities of invertebrates, and their low human visitation make them key models to study ecological and evolutionary changes in aliens' life traits (Lebouvier et al. 2011). Here we provide a rare example of coherent fingerprint of dispersal promotion across several morphological traits in *Merizodus soledadinus* (Coleoptera, Carabidae), an insect invading the Kerguelen Islands. This flightless predatory ground beetle was accidentally introduced on one occasion at the single site of Port Couvreux a century ago (Jeannel 1940), and subsequently spread and founded new populations far from this introduction point. The long-term monitoring of its spread since this single event of introduction provides a unique opportunity to address evolutionary changes during the invasion process (Lebouvier et al. 2011).

Based on the synchronic data of Laparie et al. (2010) that consist in the morphological comparison of populations of *M. soledadinus* with different residence times, we tested the idea that progressive selection for dispersal ability may have occurred along the invasion front. We expected a quantitative relationship between residence time of geographically distinct populations and dispersal-related morphology.

Materials and methods

Collection sites

In the present work, we reanalyzed the morphological data among populations of *M. soledadinus* from Laparie et al. (2010). The long-term monitoring of invasive species conducted at the Kerguelen Islands allowed to select a set of five populations with distinct residence times (Fig. 1). All individuals experienced similar abiotic conditions, as (i) they were collected under seaweeds, so that

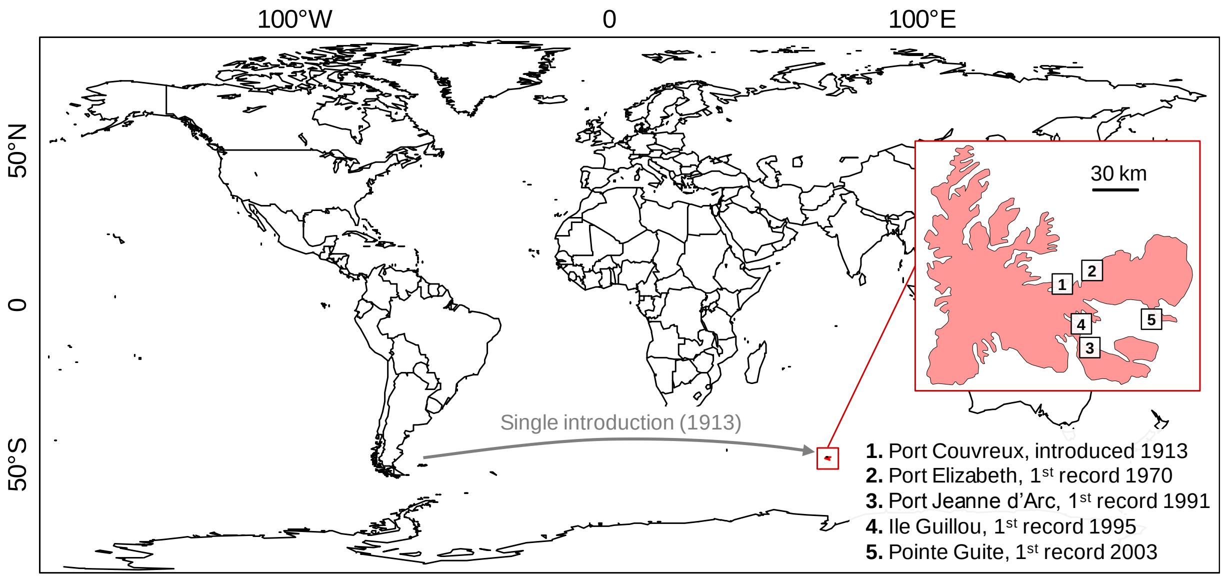

Fig. 1. Location of the Kerguelen Islands, and populations sampled with their respective estimated colonization date. For ease of visualization, small islands of the Kerguelen archipelago were not represented in the magnified panel. The maps were plotted using *R* 2.13.0.

there were strong similarities among sampled microhabitats, and (ii) the most distant sites were separated by less than 50 km, without topographic barriers to alter temperature or rainfalls (Fig. 1).

Measurements

Morphological traits and dry mass after ethanol storage were measured in 296 adults from the populations sampled (Table 1). In each population, 30 males and 30 females were measured (except in Port Jeanne d'Arc because only 26 males were in a sufficient preservation state for measurements).

Thorax size (PRONO_L and PRONO_W) is widely considered as a relevant proxy for movement ability of flying insects because of locomotor muscles (Beck and Kitching 2007), and may then be considered in walking species as well. Leg length (FEMU_1) may also be assumed as a substitute to wing size for inferring movement ability in this flightless beetle. Length of the last sternite (STER) may be associated with reproductive investment and oogenesis in females (Laparie et al. 2010). Traits related to head and mouthpart size (INTOC and PALP) might rather reflect feeding habits and size of food particles ingested in this generalist carnivore. Finally, elytron length (ELYT) and dry mass (MASS) may account as global surrogates of abdomen and body size, and indirectly reflect energy reserves.

Table 1. Quantitative traits measured in adult *M. soledadinus*. Different magnifications were used depending on the size of the appendage, resulting in theoretical precisions of 8.8 µm for ELYT, 0.9 µm for PALP, and 3.3 µm for all other traits; precision was 10 µg for MASS.

ELYT	Length of right elytron
INTOC	Interocular width
PRONO_L	Length of pronotum
PRONO_W	Width of pronotum
STER	Length of last sternite
FEMU_1	Length of metafemur (right foreleg)
PALP	Width of last article of maxillary palp
MASS	Dry mass (after 70° ethanol)

Statistical analyses

To investigate the relationship between residence time and size changes, we aimed at finding the linear combination of traits that best described the body size differences among populations. The STER parameter was dropped because it is most likely related to reproduction, and varies independently from overall body size (no evidence for a trade-off; Laparie et al. 2010). We ensured that there were no correlations higher than 0.8 among traits.

We used a Canonical Discriminant Analysis (CDA, grouping factor: population) to compute the optimal linear combination of traits that optimized the inter-group inertia (first canonical axis). The scores of individuals scattered along this axis were then extracted and tested in a linear regression with residence time. Rather than testing differences among qualitative groups like the CDA did, this analysis considered residence time as a discrete quantitative variable. The linear regression thus checked that the best morphological discrimination previously found sorted the populations in a way that fits the magnitude of their differences in residence time.

The analyses were conducted separately for males and females. Prerequisites of adequacy to normality of the residuals from all linear models computed were confirmed using Q-Q plots and Shapiro-Wilk tests. All statistical procedures were conducted with *R* 2.13.0 statistical software.

Results

In males, the linear combination of variables that best separated the populations accounted for 54.9% of the total inertia (Fig. 2a). Elytron length was the most discriminant trait, followed by traits related to head size, pronotum size, femur length, and maxillary palp diameter. The mass was also correlated to the first canonical axis, but there was a gap between its variations among populations and the variations of other traits (Fig. 2a).

The linear regression between residence time and individual scores on this canonical axis was highly significant. It confirmed the negative relationship expected between residence time and size: the younger the population, the higher the individual scores along the axis depicting multidimensional size variations (Fig. 2a).

Likewise, in females, the best canonical axis depicting size variations among populations accounted for 57.5% of the total inertia (Fig. 2b). The discriminant traits were sorted in the same order than in males, with elytron length being the most important trait and mass the least important one. However, the gap observed in males between elytron length and parameters associated with head and pronotum sizes was not found in female canonical structure. Compared to other parameters, femur length and maxillary palp diameter were less discriminant than in males, and mass was inversely correlated to other body size parameters along the canonical axis (Fig. 2b). This converse relationship was likely due to mass being the lowest in females from the third population (Port Jeanne d'Arc, 15 years of residence at the date of sampling) despite comparatively high values of ELYT, INTOC, $PRONO_W$, $PRONO_L$, and PALP (Fig. 3). Females from the most recent population (Pointe Guite) had high values for most traits, but were likely pulled towards low canonical values because they had the highest average mass in the sample (Fig. 3).

The linear regression between residence time and individual projections on

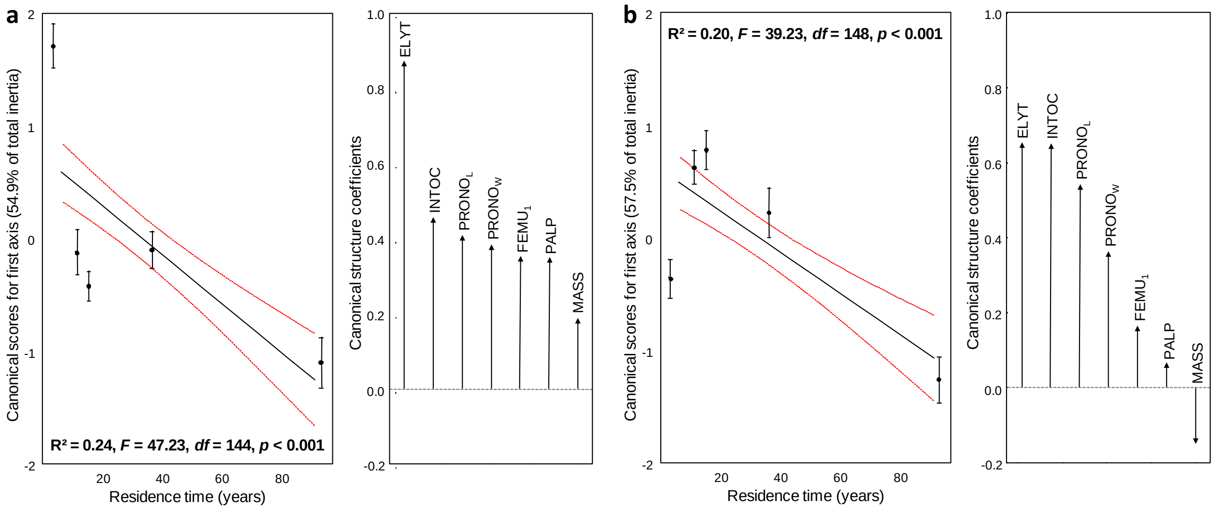

Fig. 2. Linear regression between residence time and canonical scores along the multidimensional axis that best depicts size variations among populations of males (a), or females (b). Red lines delimit the 95% pointwise confidence interval. Centroids ± SE of each population are projected onto the canonical axis according to their residence time, but both regressions were performed on individual scores (n_{males} = 146; $n_{females}$ = 150) and not centroids. Canonical structure coefficients show correlations between the seven original traits and the canonical scores; *i.e.*, they illustrate how traits are combined to construct the best canonical axis for each sex.

this canonical axis was highly significant, and consistent with the results found in male: the younger the population, the higher the individual scores along the axis depicting multidimensional size variations (Fig. 2b). This relationship was slightly weaker than in males, mostly because of females from the youngest population being pulled towards low values of the canonical axis as a result of their higher mass (see Fig. 3), as this parameter tended to offset the others along the canonical axis. Scores of females from the oldest population (Port Couvreux) were on average the lowest, as these females were characterized by low values for most size parameters while being heavier than females from Port Jeanne d'Arc (Fig. 2b; Fig. 3).

Discussion

This paper sheds light on a significant relationship between several body size parameters and range expansion in an invasive ground beetle of the Kerguelen Islands. In particular, the synchronic morphological differences among populations quantitatively matched their differences in residence time, and depicted an increasing body size gradient through the colonization history of this species. Heidinger et al. (2010) emphasized that traits such as body size, thorax mass or wing development are important for successful dispersal in insects, thereby highlighting the relevancy of using morphometrics to infer dispersal ability. Moreover, Hill et al. (1999a) found morphological changes associated with better flight ability in expanding populations of butterflies. Therefore, the pattern observed here is consistent with selection of dispersal-related traits, as it involved changes in leg length, room available for locomotor muscles (pronotum dimensions) and even body size per se.

It remains difficult to delineate intergenerational evolution and phenotypic plasticity until the underlying genetic

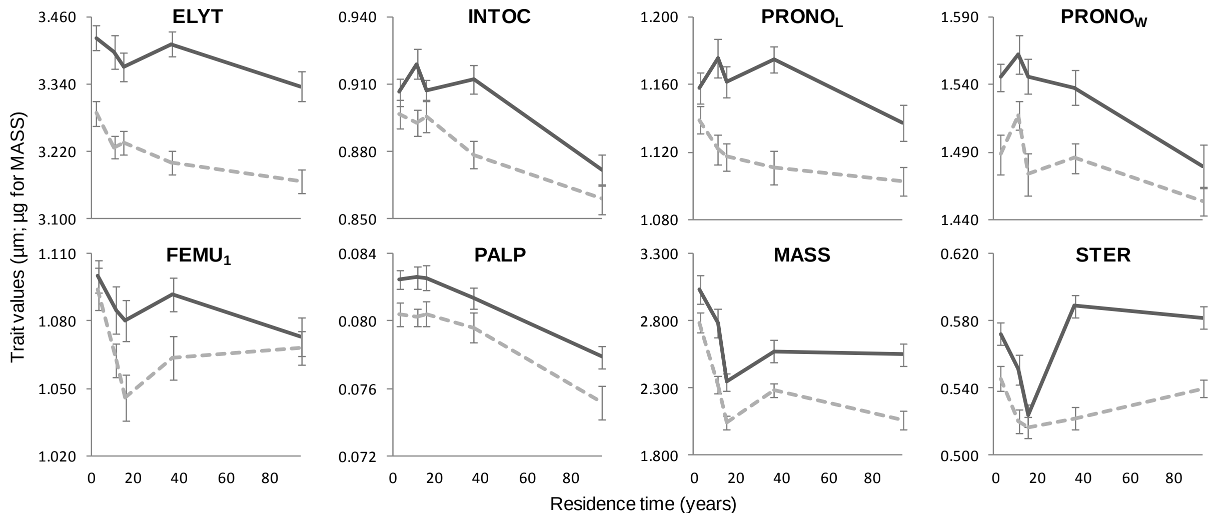

Fig. 3. Traits measured in *M. soledadinus* against residence time (mean ± SE). STER was not included in multivariate models as it was found to be independent from overall size (Laparie et al. 2010). Solid black line: females; dashed grey line: males.

architecture for the morphological differences observed is directly elucidated. This is a common issue with the observations of morphological clines in nature (Merckx and Van Dyck 2006). Nonetheless, using microsatellites, Lalouette (2009) investigated the genetic structure among several populations of *M. soledadinus* at the Kerguelen Islands, including those considered here. This author found a high allelic similarity between Port Couvreux and Port Elizabeth, the oldest populations studied here, while the most recent one, Pointe Guite, greatly differed from other populations. Albeit phenotypic plasticity and limited environmental canalization (*i.e.*, a set of processes aiming at keeping the phenotype constant in spite of environmental variation) probably partly account for the pattern observed here, they are unlikely to provide the whole explanation for the phenotypic changes observed. Indeed, at the population level, dispersers have been frequently reported as being larger or in better condition than residents (Bowler and Benton 2005). Bowler and Benton (2005) emphasized that this idea spans across a broad range of taxa, including insects (Anholt 1990; Lawrence 1987), but also mammals, birds and reptiles. More specifically, the subtle relationship between body size and tendency to disperse has been reported in some flightless carabids such as the Pterostichinae and Harpalinae (Mossakowski and Stier 1983).

Larger individuals may be more prone to disperse if a minimal amount of reserves is required to undertake dispersal, or if dispersal is associated with fasting (Bowler and Benton 2005). Indeed, intraspecific size variation has often been related to the amount of reserves that insects can store or have already stored (see review in Hoffmann and Harshman 1999). Starvation resistance is even considered size-dependant in different taxa of holometabolous insects (Arnett and Gotelli 2003; Reim et al., 2006), including females of *M. soledadinus* (Laparie et al. 2011), and can contribute towards surviving the dispersal process. Li and Margolies (1994) even observed evolution of starvation resistance as a correlated response to selection on dispersal behaviour in spider mites. Hence, larger individuals with more reserves may be more efficient in perpetuating their phenotype in new population pools by (i) actively or passively colonizing new sites, and (ii) subsequently expending energy to reproduction. The synchronic assessment of spatial selection is particularly relevant in *M. soledadinus*, as individuals all originate from a single pool introduced in 1913 at Port Couvreux, thus providing a unique benchmark to monitor changes during the invasion process (Laparie et al. 2010; Sax et al. 2007).

Positive selection for dispersal must be considered with caution, as the data do not depict a decreasing variability in young populations. However, selection for residency may also occur after successful establishment, as part of a dispersal syndrome setting dispersal and reproductive strategies to promote settlement (see Roff 1986; Zera and Denno 1997; studies in carabid species by Desender 2000; Matalin 1994). The highly sexually dimorphic variations of the last sternite are consistent with this view. Indeed, the sternite was the largest in females from the oldest populations, despite much lower values in corresponding males, as well as small overall body size. By contrast, the sexual dimorphism for this trait dramatically dropped in younger populations, with the sternite being most likely reflecting correlations with overall body size in both sexes (Fig. 3). Consistently, Crawley (1989) reported that large body size is generally thought to promote invasion success, but is partly offset by lower reproductive rates associated with costs of being larger. Our results indicate that such selection pressures are likely to act differently on both sexes, in agreement with the observations of Heidinger et al. (2010) on grasshoppers.

The variations of traits that are not associated with movement ability (those measured on head or mouthparts for instance) may reflect feeding strategies, diet breath, and regression of preferred preys. There are clues for food preferences in *M. soledadinus* (Laparie et al. 2011) that match the local extinction of some macroinvertebrate prey species in areas altered by the long-term residence of this predator (Lebouvier et al. 2011). The altered prey communities imply dietary changes towards smaller species in resident *M. soledadinus*, and may be responsible for changes in head size and morphology of mouthparts. Although Todd (1996) found no significant relationship between adult body mass of *M. soledadinus* and size of the food particles ingested, we showed that large dimensions of head (INTOC) and mouthparts (PALP) do not necessarily correlate with body mass (see Fig. 3). These parameters are presumably more tightly associated with selection of food items than body size, and their small size in old populations may reflect a less generalist diet compared to young populations that coexist with the most diverse prey communities.

Of particular interest is the population of Port Jeanne d'Arc, first recorded in 1991, which breaks to some extent the general trend, as it exhibited low values for most traits despite a comparatively short residence time (Fig. 3). Port Jeanne d'Arc is a former whaling station founded by a Norwegian company in 1908, consisting of four large residential buildings plus a barn. One of them was restored in 1977, thereby causing intense human activities and transport of materials by ships. As populations of *M. soledadinus* are particularly dense around barracks, rubbles and other human materials (Jeannel 1940), resident individuals may have been introduced with their microhabitat from a remote site. The colonization four years later of Ile Guillou may also result from human visitation, as this island is disconnected from the largest landmass of the Kerguelen Islands by a two-meter wide sea inlet. Although floating individuals may successfully cross this kind of barriers without human assistance (Renault 2011), Ile Guillou and Port Jeanne d'Arc are separated by several kilometers and showed strong genetic similarities, with the same characteristic reduction of allelic richness (Lalouette 2009). This result indicates that both populations are closely related, and experienced strong and similar founder effects. The morphological similarities between these populations corroborate their similarities in both genetic structure and residence time.

Most current approaches of dispersal evolution consider that movement ability is static in a population, but our results support the predictions of Phillips et al. (2008) that it is wrong for range expansion because of possible spatial selection and promotion of dispersers on front margins. The increasing spread rate predicted at the distribution margins (Phillips et al. 2008) is a particular issue in the case of invasive species, as it makes them more and more invasive, and can lead to underestimation of the time course of future range expansions.

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2.4. Disentangling local adaptation and phenotypic plasticity: a challenge for the future

Evolutionary changes and local adaptations are a major scenario in explaining biological invasions because novel selection regimes may indirectly promote the optimal variants of an alien species in its new environment (Facon et al. 2006; **section 1.2.4.2.**). Reduced genetic variance due to bottleneck and founder effects is commonly viewed as a limit for such optimizing selection in the invaded environment. Indeed, introductions can involve a small number of propagules, accounting for little genetic variation, and thereby representing a limited fuel for responding to selection (Facon et al. 2006, Sax et al. 2007).

However, the study of biological invasions provided numerous proofs that adaptive genetic changes can occur rapidly in the invaded habitat, and that severe population bottlenecks do not preclude subsequent rapid adaptation (Sax et al. 2007, Roderick & Vernon 2009). By contrast, bottlenecks can even facilitate rapid evolution by increasing the relative importance of genetic drift and epistatic interaction among genes, thereby promoting adaptive responses to novel selection regimes (Sax et al. 2007). As an example, the invasion of *Drosophila subobscura* in southern Chile in the 1970s resulted from the introduction of less than 15 individuals with no other known episodes of introduction (Huey et al. 2005). Yet, the species encountered a tremendous success, spreading across 12° of latitude (Huey et al. 2005). These authors reported morphological divergence from the ancestral population, and geographical clines in wing size, with underlying genetic differentiation. These results provide evidence that invasive species are unrivalled models for tackling rapid evolutionary processes, and that these changes can result in morphological clines to be addressed using morphometrics. Unfortunately, case studies with known benchmarks (the precise year in which a species was introduced to a particular location; Sax et al. 2007) like in the example of *D. subobscura* in Chile, or *M. soledadinus* and *C. vicina* at the Kerguelen Islands, are considerably rare in comparison to the number of biological invasions worldwide (Davis 2009).

Range expansion of invasive species may be assisted by colonist individuals that exhibit better motivation or success at migrating, and thus contribute towards explaining the apparition of geographical clines in morphology and genetics. Extreme cases may lead to discrete polymorphisms (Bonte et al. 2010) with winged and flightless morphotypes, such as those reported in insects by Roff (1986). Delattre (2010) emphasized that examples of more continuous morphological variations are also known, namely involving variations in body size, thorax, or wing loading (Hill et al. 1999a, Hill et al. 1999b, Merckx et al. 2006, Merckx & Van Dyck 2006). Nevertheless, it is often difficult to discriminate selection from phenotypic plasticity in nature (Merckx & Van Dyck 2006), as also illustrated here with the morphological variations observed in time and space in *M. soledadinus* and *C. vicina*.

Understanding the relative importance of the underlying mechanisms that alter morphology of invaders has become a crucial question, especially because morphology can, in turn, interact with these mechanisms. Morphometrics have proven to be a useful tool for investigating the long-term ecological interaction between alien species and their novel environments, and this approach can play a significant role in bridging the gap between phenotype and durable success of invasive species. Aside from genetics, geometric morphometrics have become one of the most frequent tools for monitoring evolutionary processes in animals, and both are increasingly combined to each other in recent studies. We believe that the case models studied in the present thesis provide a fertile ground for such integrated approaches, aiming at disentangling the forces that shape morphological patterns over time and space during biological invasions, and should be considered as future directions of the present work.

CHAPTER 3

THE ROLE OF PLASTICITY IN THE COLONIZATION OF NEW ENVIRONMENTS

Plasticity, then, in the wide sense of the word, means the possession of a structure weak enough to yield to an influence, but strong enough not to yield all at once.

— William James (*The Law of Habits, The Popular Science Monthly*, 1887)

3.1. What are the microhabitats invaded by *Merizodus soledadinus*?

3.1.1. Context

The wide distribution of *M. soledadinus* in coastal areas, which is generally covered with low herbaceous vegetation and sometimes defined as tundra, has been already reported (Chevrier et al. 1997, Brandjes et al. 1999). Field work also demonstrated that its geographical spread could reach three kilometers per year on Ile Haute (Golfe du Morbihan), where it was first observed in 1992 (Chevrier et al. 1997; Fig. 11). However, the exact microhabitats housing populations of *M. soledadinus* at Kerguelen Islands have not been described yet. In the present work, we used field-based data from our long-term monitoring programme (namely data from Chevrier 1996) to characterize the main microhabitats invaded by *M. soledadinus*. Regular trapping was conducted in several coastal habitats of Ile Haute, together with opportunistic active searches throughout other areas of the archipelago. Identifying the type of microhabitats where *M. soledadinus* occurs is crucial for understanding its spatial dynamics at the Kerguelen Islands.

3.1.2. Materials and methods

The study was conducted on Ile Haute (Golfe du Morbihan) at the Kerguelen Islands from February 1995 to July 1995, and completed from 2005 to 2009. In the first part of the study, a total of 43 pitfall traps (9 cm diameter and 4 cm height) were positioned around Ile Haute (< 5 m above sea level). Pitfall traps were opened for four days once a month, *i.e.*, six times during this study. The main characteristics of each habitat and microhabitat nearby the pitfall traps were described in Table 1 according to Hughes (1987).

In the second part of this study, numerous additional sites and habitats were examined by visual inspections from 2005 to 2009 to maximize the number of investigated habitats. Inspections were conducted in 22 distinct locations on the eastern part of the Kerguelen archipelago (Péninsule Courbet, Presqu'île Ronarc'h, Presqu'île Jeanne d'Arc, Plateau Central and islands in the Golfe du Morbihan; Fig. 11) in coastal areas under stones, timber, and decaying algae (*N* = 540 sites). Inland observations were also conducted under mammal carrions (*N* = 157 sites) and stones (*N* = 384 sites) in fell-fields (bryophytes), lowland rock fields (*Ranunculus pseudotrullifolius*, *Azorella selago* and bryophytes), tussock grasslands (*Poa* sp.), meadows (*Leptinella plumosa*, *Poa cooki*, *Deschampsia antarctica*), herbfields (*Acaena magellanica*, *Callitriche antarctica*, *Pringlea antiscorbutica*), peat bogs, cushion-carpets (*Azorella selago*, *Colobanthus kerguelensis*). Each inspection lasted 10 min per person in a total of 1081 visited sites.

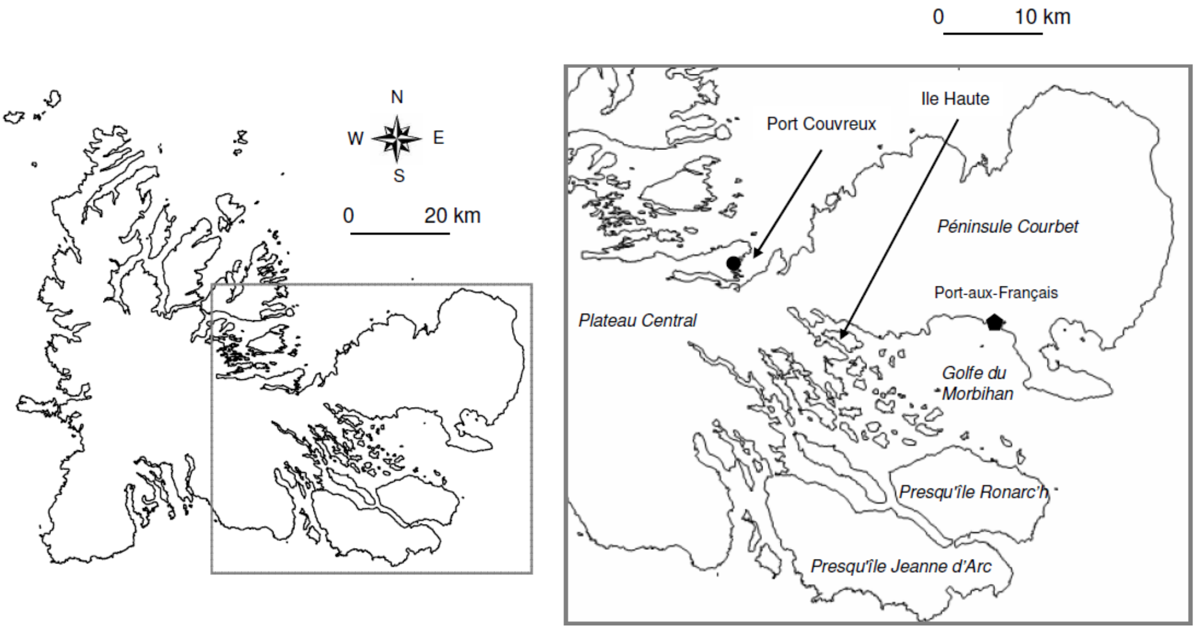

Fig. 11. Map of the Kerguelen Islands with a focus on the eastern part of the archipelago. The research station is based at Port-aux-Français, and the ground beetle *M. soledadinus* was introduced at Port Couvreux.

3.1.3. Results

Specimens of *M. soledadinus* were sampled in 24 of the 43 pitfall traps (56%; Table 1). Few *M. soledadinus* were sampled when there were herbfield and bare soil close to the traps (Habitat D), and additional inspections conducted in the second part of this study revealed anecdotic (< 5 ground beetles per 10 minutes search period) to nonexistent densities in fell-fields, rock fields, peat bogs and other low altitude habitats with a spongy soil like wet valleys (bryophytes) and meadows with *Juncus acutiflorus*. Habitats close to foreshore in scree areas with high gullies, and habitats with few plant patches, or open lawn with *Crassula moschata* or *Poa annua* and *Taraxacum* sp., were also characterized by low densities of *M. soledadinus* (< 5 ground beetles per 10 minutes search period).

Table 1. Main characteristics of the habitats where pitfall traps (N = 43) were positioned on Ile Haute and number of traps where *M. soledadinus* was sampled. "Positive traps" refer to the ratio between traps with and without *M. soledadinus*; "Trapping rate" refers to the number of individuals of *M. soledadinus* per trap per day.

Habitat	Distance to the sea (d)	Exposition	Habitat category; main plant cover	Microhabitat around traps	Positive traps	Trapping rate
A	d < 5 m	S, NE, NW	Tussock grassland and pebbles; *Poa annua*	*Poa annua*	7/15	0.80
B	5 < d < 10 m	SW	Cushion-carpet and bare soil; *Azorella selago*	Bare soil	4/4	1.00
C	d < 5 m	S, N	Meadow and pebbles; *Leptinella plumosa*	*Leptinella plumosa*	12/19	1.62
D	5 < d < 10 m	NW	Herbfield, bare soil, blocks; *Poa annua*	*Poa annua* and *Taraxacum* sp.	1/5	0.05

Specimens of *M. soledadinus* were regularly sampled in habitats A, B and C. The second part of the study revealed that they were also very often found around the strandline (372/540 observations, *i.e.*, 69%) (1) under the seaweeds and timber, (2) beneath stones in coastal areas near penguin colonies, (3) in herbfields and meadows. Interestingly, 200 meters away from the sea on Ile Guillou (Golfe du Morbihan), both adults and last larval instars have been found down to 25 cm depth, into the rhizosphere of *A. magellanica*. Yet, we found that this ground beetle was not restricted to coastal areas and could be found inland (265/541 observations, *i.e.*, 49%) (i) in the vicinity of cushion-carpets, (ii) along rivers and ponds beneath stones or mammal carrions and (iii) in fell-fields when there were some plant species (*A. selago*, *C. kerguelensis* or *Lycopodium magellanicum*) and/or bryophytes. Recent inspections in 2010-2011 revealed the occurrence of new small populations more than 315 m above sea level. For instance, 8 individuals/person/10 min were found on top of the Volcan du Diable, under stones surrounding scarce patches of *A. selago*, and even under some rocks isolated by several meters from visible vegetation. Alghouth mites, weevils, caterpillars and native spiders were found in small densities in the same habitat, *M. soledadinus* was the dominant species.

3.1.4. Discussion

The habitat distribution of *M. soledadinus* has long been thought to be restricted to herbfields of coastal areas, near the ruins and rubbles of the whaling stations and other human materials in the vicinity of its original introduction points (Jeannel 1940, 1964, Ernsting 1993, Brandjes et al. 1999). Nowadays, this ground beetle is also often found beneath stones, rubbles, and in tussock in South Georgia (Convey et al. 2010). In the present work, we found specimens of *Merizodus soledadinus* in several distinct habitats at the Kerguelen Islands, and no distinctive pattern appeared between the occurrence of this species and the main plant cover. The occurrence of *M. soledadinus* is rather governed by the structure of the communities of invertebrates (characterized by the dominance of decomposers, Vernon et al. 1998) and the moisture level of the microhabitat, an observation consistent with both field observations conducted in Patagonia (Douady C., Kaufmann B., Lebouvier M. & Renault D., unpublished data) and experimental work. Todd & Block (1997) already demonstrated the reduced tolerance of adult *M. soledadinus* to desiccation, and this species is mainly active during night to prevent desiccation (Ottesen 1990). Large numbers of specimens were sampled along strandline, under decaying seaweeds where massive amounts of organic matter is deposited. *Merizodus soledadinus* was found to mostly co-occur with massive densities of maggots of several native (*Calycopteryx moseleyi*, *Anatalanta aptera, Amalopteryx maritima*) and introduced (*Calliphora vicina*, *Fucellia maritima*) flies, springtails, caterpillars of the moth *Pringleophaga kerguelensis*, or several native weevil species, all being assumed as consistent prey organisms (see Lalouette 2009, Lebouvier et al. 2011).

Conversely, the communities of invertebrates are more depauperate in fell-fields and rock fields, and the diversity and abundance of saprophagous flies are low. Although the spiders *Myro kerguelensis* and *Neomaso antarcticus* also occur in these habitats, their densities are dramatically reduced. The same observation stands for the moth *P. kerguelensis*, as well as several species of mites, weevils, and the snail *Notodiscus hookeri*. The depauperate prey communities in these areas, alongside probable abiotic constraints, are the main reasons for explaining why *M. soledadinus* was only recorded recently, and in small populations, in fell-fields and rock fields. The presence of adults 10 to 25 cm below the ground level nearby the roots of *A. magellanica* is reported for the first time. Moisture in this microhabitat may be of prime importance for the biology of this species at the Kerguelen Islands, but it remains to be determined if the individuals reported here were exclusively teneral adults that had not spread yet, or if they can indeed inhabit this type of habitat during their whole adult life. Carabid larvae usually exhibit a cryptic behaviour and are sampled in low densities, therefore, as already hypothesized by Jeannel (1940), it is likely that larval development of *M. soledadinus* proceeds in the soil at least for the first instars.

Jeannel (1964) hypothesized that the founding population of *M. soledadinus*, naturalized along the buildings of Port Couvreux, would be prone to extinction when the last ruins would disappear. Even if this species still proliferate around human constructions nowadays, the current geographical distribution of *M. soledadinus* at the Kerguelen Islands surpasses by far the range limits of human activities, depicting the tremendous success of this invasive species. Its distribution appears to be mainly determined by (i) the ground moisture and (ii) the occurrence invertebrates species (mostly maggots, caterpillars, larvae of weevils, and springtails). Biosecurity measures have been set to mitigate accidental introduction in pristine areas not yet colonized by *M. soledadinus*.

3.2. Salinity tolerance assists the success of *Merizodus soledadinus* (ARTICLE V)

Ecological tolerance to severe environmental conditions and phenotypic plasticity are major features contributing to the success of invasive species (Lodge 1993, Moyle & Light 1996, Wilson et al. 2009b). At the Kerguelen Islands, *M. soledadinus* has long been restricted to coastal areas under sea influences (salinity resulting from spray, tide, marine-related organic matter), probably because of the abundance of prey communities. Yet, it mostly occurs in non-saline habitats such as forest litter in its native range, in Patagonia, and it was also recently found to colonize inland habitats at the Kerguelen Islands. These observations emphasize its broad ecological tolerance regarding osmotic constraint and its significance for its invasive spread. The following article aims at assessing the physiological plasticity of *M. soledadinus*, and its survival outcomes, regarding a range of saline conditions at the Kerguelen Islands. We used metabolomics to address this topic, as these novel methods constitute sensitive tools for investigating the physiological adjustments that organisms express in response to their environment.

Hidalgo, Laparie *et al. in prep.* Article V

Metabolic fingerprinting and molecular responses to salinity in the invasive ground beetle *Merizodus soledadinus* at the Kerguelen Islands

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Abstract

Salinity is an abiotic factor that impacts survival and fitness of terrestrial insects. Osmotic and ionic perturbations are induced by salinity that can be counterbalanced by accumulation of compatible solutes. The invasive ground beetle *Merizodus soledadinus* colonized coastal Kerguelen Islands, and must withstand variations in salinity levels. Interestingly, the species primary distribution is in forests and riparian zones, where salinity levels are considerably lower. In the present study, we examined the metabolic adjustments of adult *M. soledadinus* experimentally subjected to three salinity levels (0, 35, 70‰) at 4 and 8 °C. We hypothesized that this invasive ground beetle can withstand a large range of salinity conditions due to the accumulation of compatible solutes. Metabolite profile analyses revealed (i) intermediary metabolite reduction when the experiment duration lasted four weeks; and (ii) compatible solute (erythritol, alanine, glycine, proline and pipecolate) accumulation under the highest salinity conditions (70‰). The compatible solutes likely assisted in osmoregulatory processes. We also used a complementary molecular approach to assess heat shock cognate 70 (HSC70) expression variability in *M. soledadinus* at 0, 35, and 70‰ salinities, and 4 and 8 °C. HSC70 expression decreased with increasing salinity, but was not temperature-dependent.

Keywords

Biological invasions, chemical chaperone, compatible solute, insect, osmoregulation, survival

Highlights

- We determined the metabolic responses to salinity (0, 35, 70‰) in an invasive ground beetle
- Hypersaline conditions strongly impaired the duration of survival
- The amounts of several osmolytes remained very low
- Glycine was the most abundant metabolite in hypersaline conditions

Introduction

Salinity places physiological challenges on terrestrial insects, including osmotic and ionic stresses that disrupt body water volume, biochemical processes, cellular homeostasis, and ionic balance (Witteveen et al. 1987; Sowers et al. 2006). Meanwhile, several terrestrial arthropods can endure and survive saline levels up to 70–80‰ (Pétillon et al. 2008 2011) by accumulating several osmolyte classes (Yancey 2005). These osmoprotectants contribute to maintain ionic gradients among body compartments, and therefore intracellular osmotic balance (Benoit 2010). Due to the physico-chemical properties of osmoprotectants, intracellular biochemistry, and physiology are not altered. Indeed, osmoprotectants are not detrimental to macromolecules even at high concentrations, and have limited effects on pH, charge balance, and enzyme activity (Yancey 2005). These protective osmolytes include monosaccharides (glucose and fructose), disaccharides (trehalose), sugar alcohols (glycerol, inositol, sorbitol, and mannitol), quaternary amino acids (proline, alanine, glycine, taurine), and methylammonium and methylsulfonium solutes (glycine betaïne and Dimethyl-sulfoniopropionate) (Yancey 2005; Wang et al. 2008; Misra and Gupta 2005).

The intra and extracellular buildup of osmoprotectants is well known to attenuate acute hyperosmotic stress. Patrick and Bradley (2000) demonstrated that hypersalinity stress in the mosquito *Culex tarsalis* (Diptera, Culicidae) larvae was counterbalanced by high proline accumulation in intra- and extracellular compartments, and increased hemolymph trehalose levels. Osmoprotectants have also been shown to scavenge reactive oxygen species (ROS), which increase under hypersaline conditions as a result of salt-altered membrane potential (Vaidyanathan et al. 2003). Lastly, osmoprotectants were found to stabilize proteins in their natural conformation and were thus termed ‘chemical chaperones’ in reference to the well-described mode of action of other member of molecular chaperone family (Welch and Brown 1996). Modulation of intracellular levels of glycerol and proline control chaperone-mediated protein refolding in the cell (Diamant et al. 2001)

Concomitantly with the accumulation of osmolytes, heat shock proteins (HSPs) and heat shock cognates (HSC70) are synthesized in response to abiotic stress, including heat shock and salinity change (Hightower 1991; Korsloot et al. 2004). The inducible HSP70 (approximately 70 kDa in molecular mass), and constitutive HSC70 forms represent two of the main proteins from the HSP and HSC families (Tissieres et al. 1974; Liang and MacRae 1997). Inducible forms (HSP70) assist proteins in attaining native conformation following partial denaturation; constitutive forms (HSC70) are commonly detected in unstressed cells. HSC70 forms of heat shock cognates contribute the maintenance, in part, of protein homeostasis (*i.e.* abnormal protein degradation), the folding and assembly of proteins following translation, membrane translocation mechanisms, and/or cellular protein transport (Feder and Hofmann 1999). These mechanisms explain why in many insect species, HSP70 expression is up-regulated during a return to permissive conditions (Košťál and Tollarová-Borovanská 2009), and during stress, HSC70 amounts indicate if insects are capable of maintaining activities (*i.e.* transcription).

Merizodus soledadinus Guérin-Méneville (Coleoptera, Carabidae) was first described from the Falkland Islands, but its native range spans from the Falkland Islands to southern South America (Patagonia) (Jeannel 1940). A recent field survey conducted in Patagonia, together with the scarce literature available on the biology and ecology of the species, suggested that *M. soledadinus* is most often found in *Notophagous* sp. forest litter, or along rivers in valleys (Roig-Junent and Dominguez 2001; Douady C., Kaufmann B., Lebouvier M. & Renault D., unpublished data). *Merizodus soledadinus* was first sampled at the Kerguelen Islands in 1939 (Jeannel 1940). In contrast with its native range, *M. soledadinus* habitat throughout invaded regions has long been restricted to coastal areas, in herbfields of the supratidal zone, or under the foreshore (Ernsting 1993; Lebouvier et al. 2011), where the frequency of this species can be high (Laparie et al. 2010). Prey community structure characterized by the dominance of invertebrate decomposers, (Vernon et al. 1998) likely governs this distribution, as well as microhabitat moisture levels.

Typical seawater salinity ranges from 31 to 38‰ (Rudloe 1979), and can exhibit marked variability in the intertidal zone, due to daily tides, dilution from rainfalls, and evaporation, with resulting salinities up to 55 to 80‰ (Rudloe 1979). As a result, a consideration of the native ecological range of *M. soledadinus*, and its invasive success in hypersaline strandlines of the Kerguelen Islands is intriguing. Indeed, most terrestrial species from saline environments can survive in non-saline environments; however examples of species from non-saline habitats colonizing intertidal zones are more rare (Pétillon et al. 2011).

Saline stress effects are well documented in plants (Misra and Gupta 2005), in fishes (Kefford et al. 2004), in algae (Kirst 1990) and in marine arthropods (Rhodes-Ondi and Turner 2010), but few studies have been conducted on terrestrial arthropods (Rhodes-Ondi and Turner 2010; Pétillon et al. 2008). In the present study, we examined the physiological plasticity of adult *M. soledadinus* subjected to a range of experimental saline conditions (0, 35, and 70‰). We hypothesized that (i) changes in environmental salinity will result in compatible solute accumulations to adjust hemolymph osmolality and assist protein folding (chemical chaperones) in adult ground beetles; and (ii) the kinetics of these adjustments will be temperature-dependent.

Materials and methods

Study site and sampling method

Merizodus soledadinus adults were hand-collected from the Kerguelen Islands in the vicinity of Port-aux-Français research station (49°21'S, 70°13'E) in February 2010. Insects were subsequently maintained under controlled conditions at 8 ± 1 °C (R.H. of 70 ± 5%) in the dark for two days. During this 48-hours period, the ground beetles were supplied *ad libitum* with water and food. Food consisted of *Fucellia maritima* larvae (Diptera, Anthomyiidae). *Merizodus soledadinus* imagoes were then directly used to assess salinity tolerance. Individuals were starved during the experiments to avoid biasing measurements due to the presence of food in the digestive tracts. A previous study showed that negligible adult mortality occurred in *M. soledadinus* following four-week of starvation (two deaths on 28 individuals, Laparie et al. 2011). Field-fresh sampled insects, *i.e.* wild ground beetles, were also prepared.

Salinity tolerance

Merizodus soledadinus salinity tolerance was determined by placing batches of ten adults in airtight plastic boxes (diameter 8 cm, depth 6 cm), which were half-filled with local sand saturated with water. Three soil salinity level treatments were used as follows: null salinity (sand and mineral water-0‰ salt), medium salinity (sand and mineral water-35‰ salt), and hypersalinity (sand and mineral water-70‰ salt). Two temperature regimes were assessed for each of the salinities (4.0 ± 0.5 °C and 8.0 ± 0.5 °C). Local sand was washed several times with fresh non-saline water until the salinity reached a nearly null (0‰) concentration. The sand was sterilized with an autoclave, subsequently stored at -80 °C for one day before being dried at 60 °C for one week. Soil salinity was monitored with a WET sensor (2 cm deep; specific sand soil calibration) connected to a HH2 moisture meter (both instruments from Delta-T Devices Ltd., UK).

- Survival experiments

For each experimental condition (N = 6), five plastic boxes, each containing ten beetles, were used (N = 300). The survival duration of adult *M. soledadinus* was monitored every three days. Lt_{50} (lethal time for 50% of the population) was determined.

- Metabolic fingerprinting

Metabolic assays were performed on adult *M. soledadinus* exposed to three salinity conditions (0, 35 and 70‰) and two temperatures (4 and 8 °C). Metabolites were measured in whole body extracts: (i) in wild insects (T0); (ii) after two weeks of exposure to experimental conditions (T1); and (iii) after four weeks of exposure to experimental conditions (T2). For each experimental condition, ten samples were prepared, each sample comprised of a pool of three randomly collected beetles (*e.g.* sampled in three plastic boxes from each experimental condition) directly plunged in 800 µL of 70° ethanol and stored at -20 °C until analysis. A total of 130 samples were prepared.

Sample preparation and derivatization. Just after they were removed from the experimental conditions, batches of three animals were plunged in 800 µL of 70° ethanol and stored at -20 °C until analysis. The samples were vacuum-dried (Speed Vac Concentrator, MiVac, Genevac Ltd., Ipswich, England), and re-disolved in 600 µL of methanol-chloroform (2:1). Samples were homogenized using a bead-beating device (Retsch™ MM301, Retsch GbmH, Haan, Germany) at 25 Hz for 1.5 min. Then, 400 µL of ice-cold ultrapure water was added, and each sample was vortexed. Following centrifugation at 8,000 *g* for 10 min at 4 °C, 210 µL aliquots of the upper aqueous phase, which contained polar metabolites, were transferred to microtubes and vacuum-dried. The polar phase aliquots were resuspended in 15 µL of 20 $mg.mL^{-1}$ methoxyaminehydrochloride (Sigma-Aldrich, St. Louis, MO, USA) in pyridine prior to incubation under orbital shaking at 30 °C for 60 min. Following incubation, 15 µl of N-methyl-N-(trimethylsilyl) trifluoroacetamide (MSTFA; Sigma, #394866) was added, and derivatization was conducted at 37 °C for 30 min.

Metabolite analysis. We measured metabolites using a Trace GC Ultra chromatograph with flame ionization as the detector (GC-FID, Thermo Fischer Scientific Inc, Waltham, MA, USA). The injector temperature was held at 260 °C. The oven temperature ranged from 70 to 147 °C at 9 °C.min^{-1}, from 147 to 158 °C at 0.5 °C.min^{-1}, from 158 to 300 °C at 5.0 °C.min^{-1}, and remained for 3 min at 300 °C. A 30m fused silica column (DB-5, 30 m x 0.320 mm x 0.25 µm, 5%-Phenyl-methylpolysiloxane, 95% dimethylpolysiloxane) was used, with helium as the carrier gas at a rate of 1 ml.min^{-1}. One microliter of each sample was injected using the splitless mode (25:1). Randomized sample sequences were established for sample injection, and each sequence was initiated with a quality control. Standard samples consisting of 60 pure reference compounds at 200 and 300 µM concentrations were run. Chromatograms were deconvoluted using ChemStation v2.0.7, and metabolite levels were quantified using arabinose as internal standard and by comparison with individual external standards.

To ensure the reliability of our data and the accuracy of peak annotation, additional runs were conducted using a GC-MS (Dittami et al. 2011). It consisted of a CTC CombiPal autosampler (GERSTEL GmbH & Co.KG, Mülheim an der Ruhr), a Trace GC Ultra chromatograph and a Trace DSQII quadrupole mass spectrometer (Thermo Fischer Scientific Inc, Waltham, MA, USA). The injector and oven settings were identical those described above, and the column used was a 30m fused silica column (TR5 MS, I.D. 25 mm, 95% dimethyl siloxane, 5% Phenyl Polysilphenylene-siloxane).

Molecular analyses

▪ RNA isolation and cDNA synthesis

Adults were collected, sectioned into smaller pieces, and equilibrated in RNA*later* Solution (Ambion, USA). Samples were incubated overnight at 4 °C to allow thorough tissue penetration by the solution. The tissues were pelleted, and the supernatant containing the RNA*later* solution removed. Samples were transferred to -80 °C until total RNA extraction with TRIzol reagent (Invitrogen, Carlsbad, CA, USA). Samples were treated with DNase I (Ambion, USA) following the manufacturer's instructions, and quantified by spectro-photometry at 260 nm (BioPhotometer, Eppendorf, Germany). Single-stranded cDNAs were synthesized from total RNAs (5 µg) from each sample with Superscript II reverse transcriptase (Gibco BRL, Invitrogen) and a buffer containing dNTPs and Oligo(dT)$_{18}$ primer. The reaction mixture was heated (95 °C, 5 min) before adding RNAse OUT enzyme, and sterile water to a final volume of 20 µL. The reaction mixture was incubated for 50 min at 42 °C, and 10 min at 70 °C. For 5' and 3'-RACE PCR, cDNAs were synthesized from 2 µg RNA at 42 °C for 1.5 h using the SMART™ RACE cDNA Amplification kit (Clontech) with Superscript II reverse transcriptase (Gibco BRL, Invitrogen), 5' or 3'-CDC-primer, and SMART II oligonucleotide.

▪ PCR and cloning of cDNA in *M. soledadinus*

PCR was carried out in 100 µl volume reactions, including 10 mM KCl, 6 mM ammonium sulfate, 20 mM Tris-HCl (pH 8), 2.5 mM $MgCl_2$, 2.5 units of High Expand Fidelity DNA polymerase (Boerhinger Mannheim), and 25% of the cDNA (see previous section). In addition, 1 µM degenerate or specific primers (Table 1), and 0.8 mM each dNTP was added. Following an initial denaturation (94 °C, 5 min), the PCR amplification profile included 5 cycles of denaturation (94 °C, 1 min); annealing (55 °C, 1 min); and elongation (72 °C, 1 min). The reaction was subsequently extended for 30 cycles with an annealing temperature of 45 °C. For RACEs, we used 2 µL of 5'- or 3'-RACE-ready cDNA with Universal Primer Mix (UPM, Clontech) as the forward anchor primer, and a specific reverse 5'- or 3'-RACE primer for each gene (Table 1). Touchdown PCRs were performed using a hot start cycle profile, initiated by 1 min at 94 °C; followed by five cycles of 30 s at 94 °C, 3 min at 72 °C; five cycles of 30 s at 94 °C, 30 s at 70 °C, and 3 min at 72 °C; 25 cycles of 30 s at 94 °C, 30 s at 68 °C, and 3 min at 72 °C; and a final step of 10 min at 72 °C. PCR products were gel purified (Nucleospin Extract II, Macherey-Nagel, Düren, Germany), and cloned into pCRII-TOPO plasmids (Invitrogen, Carlsbad, CA, USA). Plasmids were isolated by minipreps (Nucleospin Plasmid, Macherey-Nagel, Düren, Germany), and sequenced (GATC Biotech, Marseille, France). 5'- and 3'-RACE sequences were combined to generate full-length cDNA.

- Reference gene selection and primer design

The expression of HSC70 and four reference genes (Actin, RPL8, RPL13 and Proteasome) were analyzed for *M. soledadinus*. Based on insect genes found in databases, degenerate primers were designed for all genes using the Eprimer3 software (mobyle.pasteur.fr/cgi-bin/MobylePortal/portal.py?form=eprimer3), with conserved protein domains (Table 1). The resulting amplicons were cloned and sequenced as described above. The corresponding sequences were deposited in GenBank: Proteasome (Accession № JN811144), RPL13 (Accession № JN811145), HSC70 (Accession № JN811146), Actin (Accession № JN811147), and RPL8 (Accession № JN833715). Subsequently, specific qPCR primers were designed, and optimal primer annealing temperatures was optimized using standard PCR tests.

- qPCR

Real-time quantitative PCR reactions were performed on the LightCycler480 Detection System (Roche Applied Science, France). Each reaction consisted of 6 µL Absolute Blue SYBR Green Fluor (Thermo Scientific, Waltham, MA, USA), 4 µL cDNA (25ng.µl^{-1}), 1 µL of each primer (10 µM), and 1 µL of sterile water. The PCR program consisted of an initial denaturation (95 °C, 5 min), then 40 cycles of 95 °C for 10 s, 60 °C for 15 s, 72 °C for 15 s. Each run included a negative control (water) and a five-fold dilution series of pooled cDNA (from all conditions) which produced a standard curve that confirmed high PCR efficiencies (90-100%). Each reaction was run in triplicate (technical replicate) for the three independent experiments. Expression levels were analyzed with LightCycler®480 software (Roche), and the cycle threshold values (Ct values) were determined for reference and candidate genes. The average Ct value of each triplicate reaction was used to normalize the candidate gene expression level to the geometric mean of the reference gene's level in Q-Gene (Simon 2003).

- Determination of the best reference gene and data analysis

The average Ct value of each triplicate reaction was used for subsequent analysis with the BestKeeper program in order to determine the best reference gene. This approach relies on the principle that the expression of a perfect reference gene should be identical in all samples and experimental conditions. In our conditions, the Actin gene was considered as displaying consistent expression in *M. soledadinus* and therefore was determined to be the best reference gene for this species.

Statistical analysis

Using *Minitab* 12.2 statistical software, probit regression models and Pearson correlation

Table 1. Nucleotide sequences of primers used in polymerase chain reaction and 5'-3' RACE for the amplification of reference genes and HSC70 sequences in *M. soledadinus*.

Primer	Direction	Sequences (5’-3’)	Used for…
HSP70_degF1	FOR	AAGGTGGARATCATCATCGCCAAYGAYCARGG	RT-PCR
HSP70_degR3	REV	AGGTTGTTGTCCTTGGTCATYGC	RT-PCR
HSP70_qF1	FOR	AAAATGAAGGAAACCGCAGA	qPCR
HSP70_qR1	REV	TCACCGACTCCCTTTTTGTC	qPCR
HSP70_Race_F1	FOR	TATGTGGCATTCACGGACACAGAA	3’ RACE
HSP70_Race_R1	REV	CATTTTTGTAAGAACCATTGAGCTAACTTCTTCGGG	5’RACE
RPL8_degF1	FOR	ATGGGTCGWGTVATHCGWGCTCA	RT-PCR
RPL8_degR1	REV	CCRGTCCTRCGRGCRGCRATRAGACC	RT-PCR
RPL8_qF1	FOR	TGCCTCTGTTGCTTGGATGGTA	qPCR
RPL8_qR1	REV	ATGCCTGTGGGTGCTATGC	qPCR
RPL13_degF1	FOR	CCDAATGGNATTTCCAYAAGGATTGGCAAMR	RT-PCR
RPL13_degR1	REV	GTVACATCMTCMGGATTYTCWGCAGCATCYTT	RT-PCR
RPL13_qF1	FOR	AATTGAACGCACAGGGACTC	qPCR
RPL13_qR1	REV	TTGAATTCCTTTTCGGCATC	qPCR
Actin_degF1	FOR	ATGTGCAARGCCGGYTTCGCCGGBGA	RT-PCR
Actin_degR1	REV	ACRGAGTAYTTCCTYTCKGGKGGAGCRATGA	RT-PCR
Actin_qF1	FOR	GAATCCATGAGACCACCTAC	qPCR
Actin_qR1	REV	AATCCAGACACTGTACTTCC	qPCR
Actin_Race_F1	FOR	ATGTGCAAGGCCGGTTTCGCCGGT	3’ RACE
Actin_Race_R1	REV	GGTGTGATGCCAGATCTTTTCCATATCATCCCA	5’RACE
Proteasome_qF1	FOR	CCGGACTAACATGGCAAACT	RT-PCR + qPCR
Proteasome_qR1	REV	TGCGATTTGCACGAAATAAA	RT-PCR + qPCR

tests were processed to measure the Lt_{50} of *M. soledadinus* under each experimental condition.

Metabolic differences among the 13 following classes of samples were evaluated: wild ground beetles, and ground beetles exposed at 4 °C (0, 35 and 70‰ salinities) and 8 °C (0, 35 and 70‰ salinities) for two and four weeks. Among sugars, polyols, and amino and organic acids, 41 compounds were identified; therefore multivariate methods were employed for data analysis. Compounds beyond detection limits were eliminated from the analysis. Compounds exceeding a 80% correlation with other compounds were discarded to avoid redundancy and an overestimate in differences among classes in multivariate analyses. Using log-transformed data, we then performed MANOVAs to address physiological differences among the 12 classes of individuals exposed to controlled conditions. Wild individuals were excluded from these MANOVAs to ensure that the differences found did not result from the contrast usually found in the literature between wild individuals and individuals maintained under controlled conditions. As the *p*-value for the interaction between exposure duration and temperature was 0.057 (rounded in Table 2), we decided to analyze 4 and 8 °C experiments separetely.

Class separation was subsequently investigated in Linear Discriminant Analyses (LDA). The wild individuals were included in these analyses so that the physiological dynamics from T0 (natural conditions) to T2 (four weeks of exposure to controlled conditions) has been addressed. Statistical significance of LDAs was checked by using permutation tests (10,000 permutations). Statistical procedures were conducted with *R* 2.13.1 statistical software (R Development Core Team 2008).

ANOVA and *post hoc* Mann-Whitney U tests were performed on molecular data using *STATISTICA* 7.0 (Statsoft).

Results

Survivorship experiments

Lethal times for 50% of the population (Lt_{50}) did not differ between thermal conditions (4 and 8 °C). Following four weeks of the experiment, non-saline conditions did not result in mortality. Lt_{50} was significantly reduced with increased salinity levels, from 27.0 ± 2.1 days at 35‰ to 19.5 ± 1.4 days at 70‰ at 4 °C; and from 21.4 ± 1.3 days at 35‰ to 15.9 ± 1.7 days at 70‰ at 8 °C ($p < 0.05$).

Metabolic profiles

Physiological differences were detected among groups of individuals depending on saline conditions, temperatures, and exposure durations (Table 2).

- Responses to saline conditions at 4 °C

The metabolic profiles of individuals maintained at 4° C significantly differed among the three saline conditions (F_{46} = 1.51, $p < 0.05$), and over the course of the experiment (F_{23} = 2.61, $p < 0.001$). However, the metabolite level changes over time were not significantly different among the three saline conditions (no interaction, F_{46} = 1.08, $p > 0.05$). The total compound concentrations (two- and four-week ground beetles pooled) ranged from 108.0 ± 10.7 $nmol.mg^{-1}$ of dry mass (on average ± SE) in ground beetles exposed at 0‰ to 168.6 ± 23.5 $nmol.mg^{-1}$ of dry mass at 70‰, with ground beetles exposed at 35‰ having an intermediary concentration of 136.2 ± 16.1 $nmol.mg^{-1}$ of dry mass.

The groups (including wild individuals) showed significant ($p < 0.001$) separation in the Linear Discriminant Analysis (LDA) (Fig. 1). The first axis (LD1) accounted for 42.0% of the total inertia, and the between-class inertia was 6.2 times higher than the within-class inertia. LD1 was primarily characterized by variation in erythritol, alanine, proline, and to a lesser extent, pipecolate, glycine and ethanolamine, sorting salinity levels from 0 to 70‰ by increasing concentrations of the three metabolites (ESM1). At 70‰, the concentrations of compounds were higher in four-week relative to two-week exposed individuals.

The second axis (LD2) accounted for 24.7% of the total inertia, and the between-class inertia was 4.1 times higher than the within-class inertia. LD2 was primarily characterized by variation in gluconolactone, mannose, glycerol, glucose, putrescine, and citrate. These metabolites had the highest levels in wild ground beetles, and the lowest levels in four-week exposed ground beetles. Differences due to exposure duration increased with increased salinity.

Table 2. Results of the MANOVA performed in adult M. soledadinus for the effects of salinity, duration of exposure, temperature, and their interactions. All numeric data were rounded to two decimal digits. Pillai trace was multiplied by 10. Significance codes: *< 0.001* "***"; *< 0.01* "**"; *< 0.05* "*"*;* not significant "*n. s.*"

Data set	Effect	F_{df}	Pillai	*p*
Laboratory individuals	Salinity	2.47_{46}	9.11	***
(*N* = 100 samples)	Duration	2.26_{23}	4.37	***
	Temperature	5.47_{23}	6.52	***
	Salinity:Duration	1.43_{46}	3.30	0.07
	Salinity:Temperature	0.96_{46}	4.94	0.30
	Duration:Temperature	1.71_{23}	3.29	0.06
	Salinity:Duration:Temperature	0.59_{46}	1.70	0.86

▪ Responses to saline conditions at 8 °C

Four-week exposed individuals at 70‰ salinity were dead and were thus excluded from the analysis. Differences in metabolic profiles among ground beetle groups exposed to the three saline conditions (F_{46} = 2.20, $p \leq 0.001$) were detected over the course of the experiment (F_{23} = 9.20, $p \leq 0.001$). However, the metabolite level changes over time were not significantly different among the three saline conditions (no interaction, F_{23} = 1.83, $p > 0.05$). The highest total compound concentrations (two- and four-week pooled) were found in ground beetles exposed at 70‰ (165.5 ± 40.6 nmol.mg^{-1} of dry mass on average ± SE), followed by those exposed at 35‰ (122.7 ± 13.3 nmol.mg^{-1} of dry mass) and 0 ‰ (104.4 ± 10.5 nmol.mg^{-1} of dry mass).

The groups, including wild ground beetles, exhibited significant ($p < 0.001$) separation in LDA (Fig. 2). The first axis (LD1) accounted for 45.9% of the total inertia, and the between-class inertia was 7.3 times higher than the within-class inertia. LD1 showed separation in the three salinity levels, and the two exposure durations at salinity levels of 35‰. The increased exposure duration at 35‰ induced increased concentrations of alanine, pipecolate, galactolactone, proline, erythritol, glycine and mannitol and decreased levels of ornithine, gluconolactone, citrate, putrescine and succinate (ESM1). Two-week exposed ground beetles at 35‰ overlapped with 0‰ samples, and two-week exposed ground beetles at 70‰ had similar metabotypes with four-week exposed ones at 35‰.

LD2 accounted for 30.6% of the total inertia, and between-class inertia was 5.5 times higher than within-class inertia. LD2 mainly discriminated wild samples for experimental samples. Decreased levels of trehalose, glycerol, fructose, mannose, citrate, gluconolactone, and putrescine characterized starved ground beetles. In a lesser extent, LD2 separated two-week exposed ground beetles at 70‰ and four-week exposed ones at 35‰.

Molecular analysis

▪ HSC70 sequence description

Merizodus soledadinus HSC70 full-length cDNA (accession number JN811146) is comprised of 2313 nucleotides (660 amino acids), including a 1980 nt coding region, and a 222 nt 3’ UTR with a polyadenylation signal sequence (AATAAA) at position 2244.

▪ Expression analysis of *hsp70* genes

Changes in HSC70 expression levels under different treatment effects were assessed in

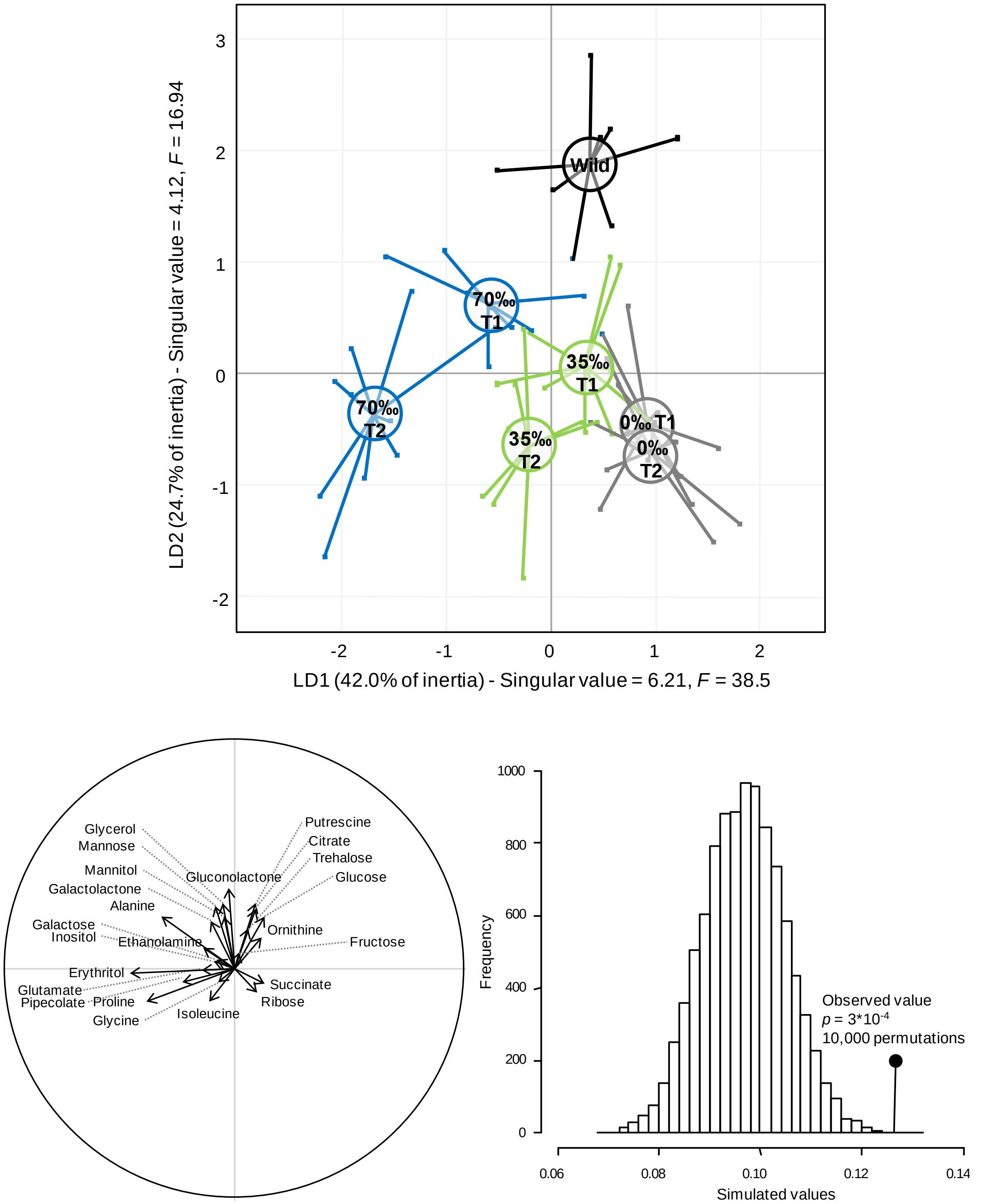

Fig. 1. Projection onto the first discriminant plane of the 63 samples of individuals maintained at 4 °C. The singular values are the ratio of between-class and within-class inertias. The lines link the samples (three individuals per samples) to the centroid of their class. Black: wild individuals, grey: 0‰ salinity; green: 35‰ salinity; blue: 70‰ salinity. The correlations circle depicts the normed relation (from -1 to 1) between each compound and linear discriminant axes. The third panel shows histograms corresponding to the permutation test compared to the observed value for the LDA, as well as its statistical significance.

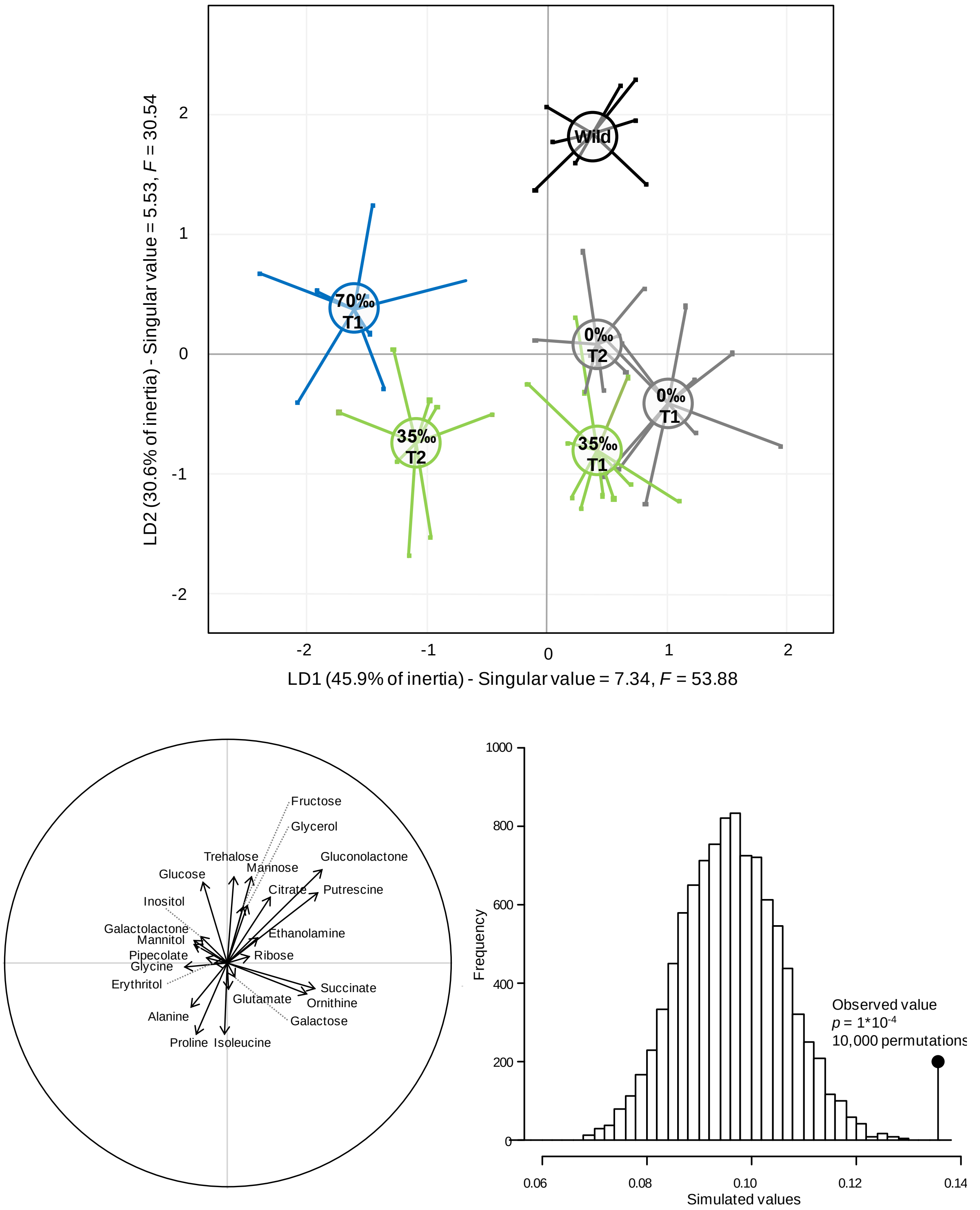

Fig. 2. Projection onto the first discriminant plane of the 53 samples of individuals maintained at 8 °C. The singular values are the ratio of between-class and within-class inertias. The lines link the samples (three individuals per samples) to the centroid of their class. Black: wild individuals, grey: 0‰ salinity; green: 35‰ salinity; blue: 70‰ salinity. The correlations circle depicts the normed relation (from -1 to 1) between each compound and linear discriminant axes. The third panel shows histograms corresponding to the permutation test compared to the observed value for the LDA, as well as its statistical significance.

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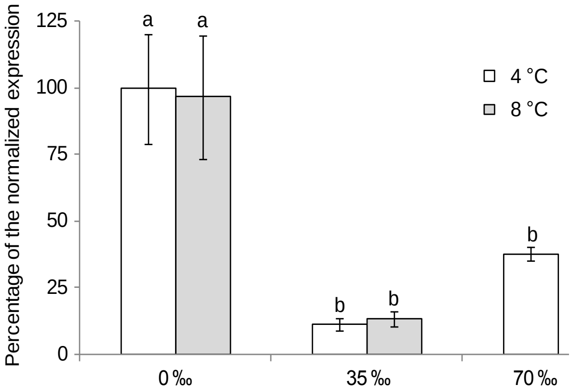


Fig. 3. Levels of mRNA HSC70 in adult *Merizodus soledadinus* exposed for two weeks at salinities of 0, 35 and 70‰ at 4 °C (white bars) and 8 °C (grey bars). Expression levels of HSC70 were normalized to the corresponding Actin abundance and expressed as the ratio to control (4 °C, 0‰), considered as equal to 100. All amplifications were reproduced in triplicate and values corresponded to the mean normalized expression (± SEM) of at least 3 independent samples. To compare data, Mann–Whitney Test was used and the letters above the bars indicates the significant differences (α = 0.05) between the mean normal expressions of control (a) and others conditions (b).

M. soledadinus using real-time PCR. Four-week exposed individuals at 70‰ salinity were dead and were thus excluded from the analysis. Three salinity conditions (0, 35 and 70‰) at 4 and 8 °C following two weeks of treatment were tested (Fig. 3). A significant difference was observed (Mann–Whitney Test, $p < 0.05$) for both thermal conditions, with a significant reduction in expression levels at 35 and 70‰ compared to 0‰. The decreased differential expression between 0 and 35%, and 0 and 70% salinities reached 8.6-fold to 2.6-fold, respectively at 4 °C, and 7.3-fold to 3.7-fold, respectively at 8 °C. Furthermore, HSC70 expression levels between the two temperature conditions (4 and 8 °C) were not significantly different, suggesting the gene was not affected by thermal, but by salinity stress.

Discussion

Survivorship experiments

The present study showed that increased salinity reduced survival duration in adult *M. soledadinus*. Mortality occurred up to three times sooner compared to control beetles, where Lt_{50} was approximately 60 days (Laparie et al. 2011). However, survival at moderate (35‰) and extreme (70‰) saline conditions remained higher than for adult *M. soledadinus* floating on seawater (Renault 2011), which is likely to have resulted in higher osmotic stress. The deleterious salinity effects on biological and physiological functions may have been associated with aerial salt concentrations in the airtight boxes, resulting in body water loss (Nentwig 1987). Under saline conditions, insect body water is usually lost during respiratory transpiration and passive body water diffusion occurs from oral and anal openings.

Thermal conditions had a slight yet additional adverse effect on survival duration. Overall insect activity is driven by environmental temperature, and a 1 °C rise in environmental temperature increased *M. soledadinus* adult metabolic rate by approximately 35% (Lalouette et al. 2011; Todd 1997). Thus, any increase of the temperature increases the metabolic rate and the amount of salt ingested by the ground beetles (by gas exchange and drinking from moist sand), and consequently heightens the negative effects of salinity.

Metabolic profiles

▪ *Evolution of metabolic response*

Metabolic changes were found among the experimental conditions, and resulted from starvation and exposure to saline conditions. Similar metabotypes were found in ground beetles exposed for two and four weeks at 0‰. The levels of several intermediate metabolites of glycolytic and energy producing pathways are usually decreased during starvation. We found slight but significant signs of energetic metabolism reduction over the course of the experiments, with mannose, fructose, glucose, and citric acid levels being reduced in *M. soledadinus*. Reductions in sugar concentration, and more notably monosaccharide and monosaccharide-P pools, which propagate to acid compounds from the tricarboxylic acid cycle (TCA), are common adaptations to salt stress in plant species (Sanchez et al. 2008). Even if monitoring respiration activity would have been necessary to ensure a reduction in catabolic rate, decreased amounts of circulating hexoses and citric acid were consistent with the presumption of a slowdown in energetic metabolism. As this predatory ground beetle relied on fatty body stores (Laparie et al. 2011) after two- and four-week exposures to the experimental conditions, it is likely that triglyceride and glycogen stores were partly depleted.

The continued reduction in gluconolactone levels, which are comprised of multiple water-attracting hydroxyl groups, likely prevented any potential for osmoprotection. Gluconolactone could result from the degradation of gluconolactone-6-phosphate produced *via* the pentose phosphate pathway. The pentose phosphate pathway is typically elicited in insects exposed to harsh environmental conditions, as it gives rise to several compatible solutes (Storey and Storey 1991; Košťál et al. 2004), and is a major reductant source (NADPH) (Kruger and von Schaewen 2003).

▪ Exposure to increased salinity levels leads to accumulation of compatible solutes

Higher total metabolite concentrations (which notably exhibited similar levels at 4 and 8 °C) sorted the ground beetles from 0 to 70‰, which supports the hypothesis that physiological changes occurred in response to hypersaline conditions. Temperature intensified the influence of hypersaline conditions on metabolic signatures, and similar metabolic signatures were observed in individuals exposed to 70‰ salinity for two weeks, and four weeks at 35‰ salinity. In addition, increased salinity and duration of exposure resulted in a stronger differentiation of the metabotypes of the ground beetles, with a progressive osmo-induced accumulation of alanine, erythritol, glycine, pipecolate, and proline. We cannot exclude that this strong metabolic signature differentiation in *M. soledadinus* at 70‰ could have resulted, at least partially, from selective adult mortality with high capability to survive under hypersaline conditions.

The accumulation of compatible solutes represents a typical plastic response of halotolerant and halophilic arthropods well adapted to environmentally saline varied conditions (Benoit 2010; Martins and Santos 1995). In plants and bacteria, the intracellular level of pipecolate has been closely related to an increase in extracellular osmotic pressure (Moulin et al. 2002); erythritol was also slightly accumulated in cells of fungi (Kogej et al. 2007). In the present work, the amounts of these two osmolytes are very low, and likely contributed little to osmoregulation processes under hypersaline conditions. Amino acids represent abundant low molecular organic substances in insects' hemolymph (Renault et al. 2006; Storey et al. 1981), and accumulation of these compounds usually accompanies osmoregulatory adjustments under saline conditions (Edwards 1982). In the present study, the pooled concentrations of alanine, glycine, and proline varied from about 46 nmoles.mg^{-1} dry mass at 0‰ at 4 and 8 °C to about 102 nmoles.mg $^{-1}$dry mass at 70‰ at both temperatures. The variations of these three amino acids likely explaining the total increase in osmolyte concentrations among saline treatments.

Increased proline is the most common response in salt-exposed plants, and has already been linked to osmotic adjustments at the cellular level when environmental salinity increases (Misra and Gupta 2005; Larher et al. 2003). Due to its physico-chemical properties, proline catches hydroxyl radicals induced by water deficits (Yancey 2005). It has also been shown that this amino acid is involved in the protection of cytosolic and cellular enzyme structure during tissue dehydration and may also act as a chemical chaperone (Sanchez et al. 2008). Even at low amounts, proline can activate the molecular chaperones (Diamant et al. 2001). Of particular note is alanine accumulation, which characterized a metabolic signature in four-week exposed ground beetles at 4 and 8 °C. Similarly, significant alanine accumulation was reported in salt-exposed Collembola (Witteveen et al. 1987), spiders, polychaete (Hoeger and Abe 2004) and mosquitoes (Patrick and Bradley 2000), in some plants (Garnett et al. 2002), or algae (Dittami et al. 2011). This reported salt-induced accumulation therefore appears as a common response among invertebrate taxa, and more particularly in osmotic conformer organisms.

Glycine was the most abundant metabolite in the ground beetles exposed to hypersaline conditions. We have no direct evidence of the potential role of glycine in protection against osmotic stress in adult *M. soledadinus*, but osmo-induced accumulation of this amino acid has already been reported in other invertebrate species (Siebers et al. 1972; Yancey 2001). This amino acid has been suggested to play a role as an organic osmolyte, assisting the regulation of cellular osmotic pressure (Yancey 2005). In addition, glycine accumulation has a beneficial effect on enzyme activity, and as such, confers a partial protection against NaCl (Pollard and Wyn Jones 1979). Salinity-dependent accumulation of glycine in adult *M. soledadinus* could play a role in osmoregulatory processes [by assisting retention of body water by osmotic forces], together with a non-osmotically function [by protecting macromolecules and more particularly enzymes].

HSC70 and environmental stresses

▪ Characterization of HSC70 sequences

Several structural characteristics have been proposed that differentiate constitutive and inducible isoforms. Some authors reported the cytosolic eukaryotic HSC70 has GGMP repeats in the C-terminal region (Wu et al. 2008). Our analyses detected three motifs at position 616, 624, and 628 in the *M. soledadinus* HSC70 (MsolHSC70) form. Finally, the 38 HSP70 and HSC70 protein sequences were allied in separate lineages in our phylogenetic tree (ESM2), and MsolHSC70 was included in the HSC70 group. Despite the difficulty in identifying the isoform of HSP (inducible or constitutive), our results indicated we cloned a *M. soledadinus* HSC70 form.

▪ Impact of salinity on MsolHSC70 expression

To date, few studies have examined the correlation between HSC70 expression and salt stress, and to our knowledge, this is the first report in insects. Lu et al. (2010) found HSP70 down-regulated protein or mRNA expression when fishes were transferred from freshwater to brackish water, congruent with the results of our study. Lu et al. (2010) obtained a partial HSP70 nucleotide sequence, without the 5' and 3' end. Therefore, it was not possible to determine if the HSP70 form was constitutive or inducible. However, because the HSP70 form was observed constitutively in control water (freshwater), we assumed their HSP70 was a constitutive form. The variation in protein expression appeared consistent with the MsolHSC70 variation observed in our study.

Chronic exposure to saline conditions of the Black Sea Bream (*Mylio macrocephalus*) also appeared to modulate HSP70 expression (Deane et al. 2002). A decrease in HSP70 protein levels was observed in juvenile livers exposed to intermediary saline conditions (12 and 33‰) compared to juvenile fish exposed to hypoosmotic water (6‰ salinity), and to hypersaline water (50‰). A similar HSC70 expression profile was observed in adult Black Sea Bream livers (Deane et al. 2004), and HSP70 expression was stable in the same tissue. Interestingly, in the marine crab *Portunus trituberculatus* and the mollusk *Crassostrea honkongensis*, HSP90 down-regulation was observed following salt stress (Zhang et al. 2009), suggesting global involvement of HSP family members in salt stress response.

Cumulatively, the previous results indicated salinity fluctuations influenced HSP70 family member expression (*i.e.* HSC70 form). Surveys of HSC70 during stress allowed us to determine if insects maintain activities (*i.e.* transcription) (Košťál and Tollarová-Borovanská 2009). In our study, the MsolHSC70 form served as a biomarker for saline stress, and would be a useful tool for monitoring salinity fluctuations in the *M. soledadinus* natural environment. Interestingly, comparisons of metabolic and molecular analyses resulted in two different responses to salt stress at the same salt concentration (35‰). These results suggest that the HSP regulatory mechanisms were saturated when the salinity levels reached 35‰.

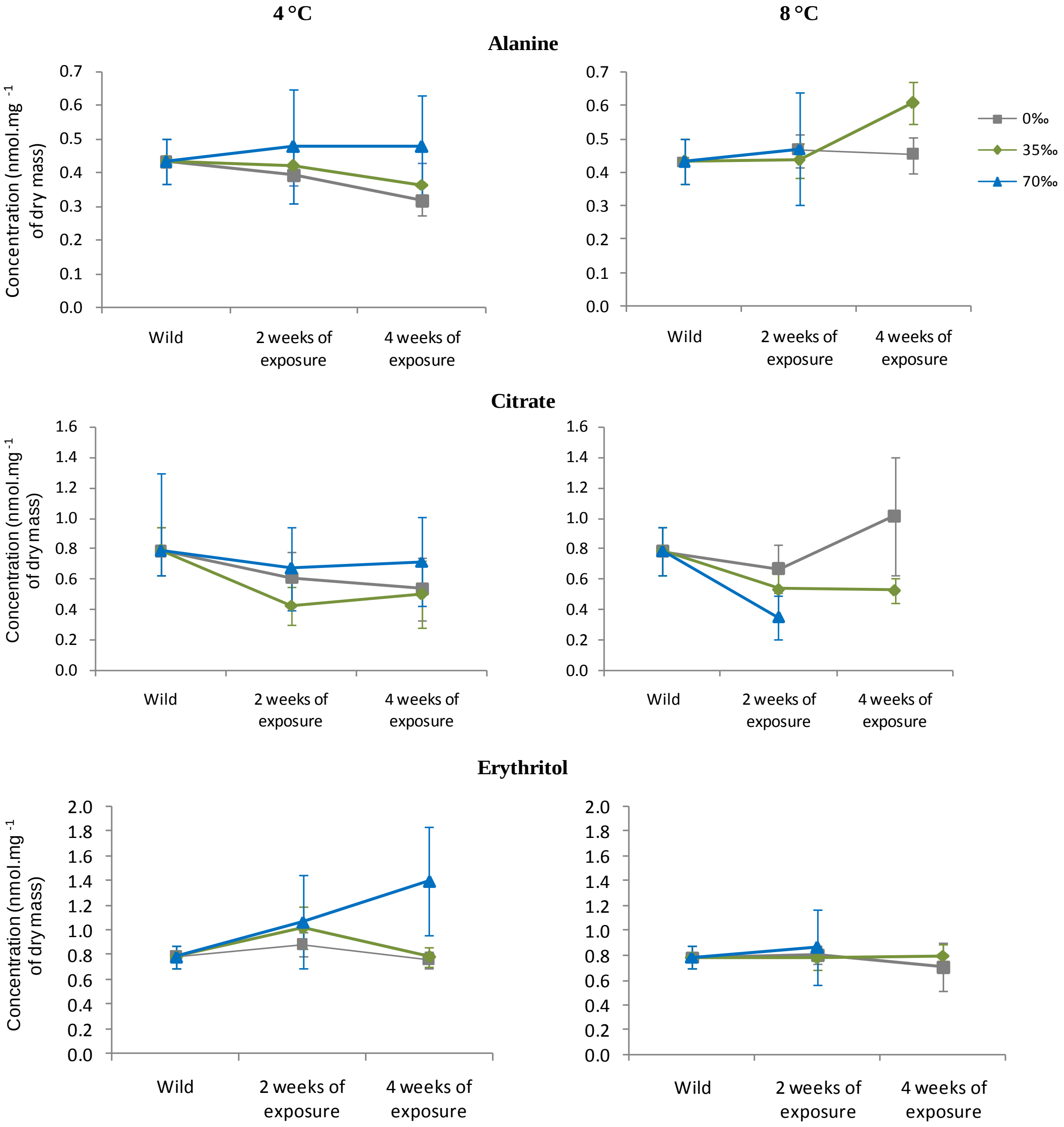
Conclusion

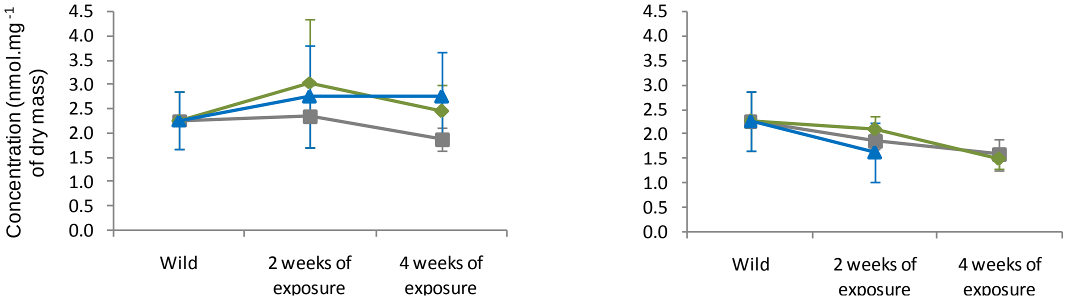
Our results did not support any role for the trehalose, a sugar usually employed as a compatible solute in insects to cope with exposure to desiccating or saline conditions. Dependent on osmoprotectant amount, the metabolites acted as osmoprotectants, served to adjust hemolymph osmolality, and for the management of carbon and reductant power. In the present study, glycine and proline were the most abundant metabolites, and exhibited osmo-induced accumulations. It is however hard to ensure the exact role played by glycine during saline exposure. Gagneul et al.(2007) already concluded that not all organic osmolytes have effective osmoregulatory functions to mitigate salinity effects, and some of them, even if accumulated, only play minor role in salinity tolerance. To conclude, we found significant changes in only few metabolites (mainly proline, glycine, and alanine) among saline treatments. However, the resulting increase in total concentration of these potential osmoprotectants was likely to modulate the ground beetles' survival on extreme saline substrates. The importance of other processes, including partial dehydration, accumulation of inorganic osmolytes, or food preferences that may assist osmoregulatory processes in this ground beetle may thus be examined in further studies.

Acknowledgements This research was supported by the Institut Polaire Francais (IPEV, programme 136 coordinated by Marc Lebouvier), the CNRS (Zone-Atelier de Recherches sur l'Environnement Antarctique et Subantarctique), and the Agence Nationale de la Recherche (ANR-07-VULN-004, Vulnerability of native communities to invasive insects and climate change in sub-Antarctic Islands, EVINCE).

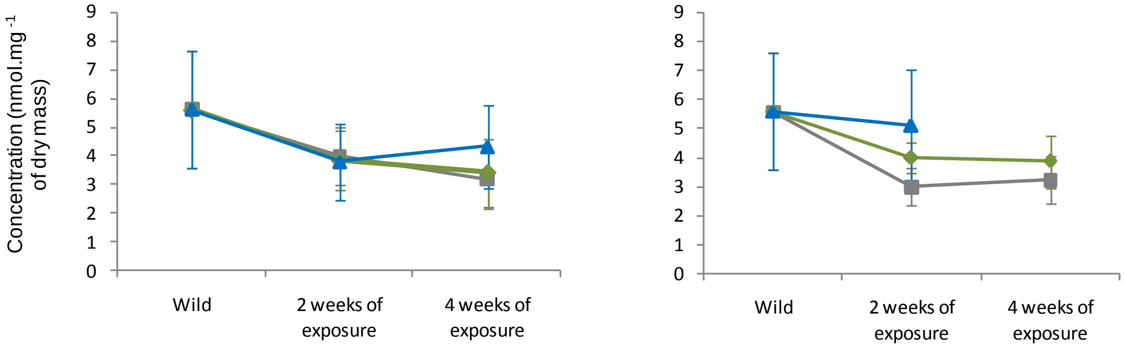
Electronic supplementary material

ESM 1. Temporal variation of compound concentrations (nmol.mg^{-1} of dry mass) ± SEM at 0, 35 and 70‰ in wild individuals and individuals exposed for two (T1) and for four weeks (T2) at 4 and 8 °C.

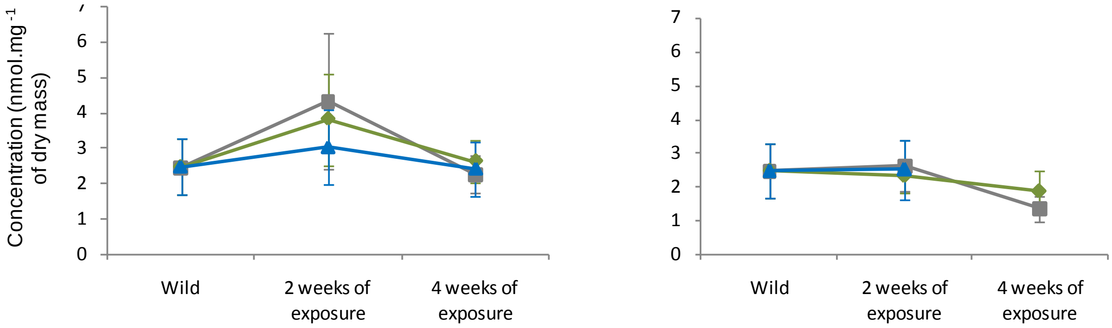




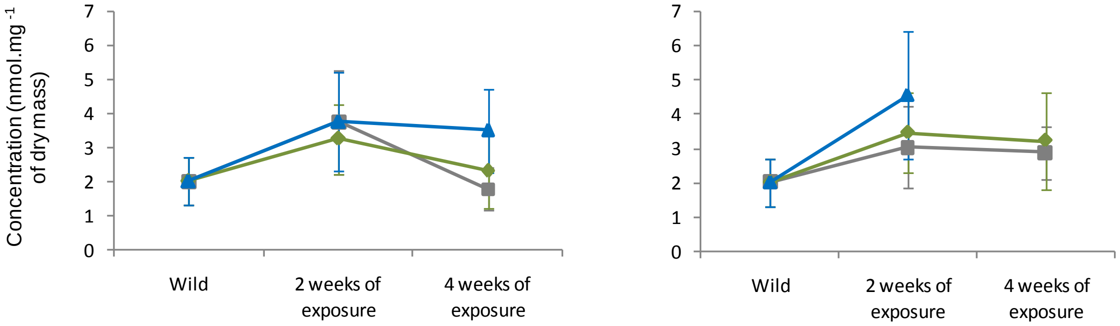
Ethanolamine



Galactonolactone

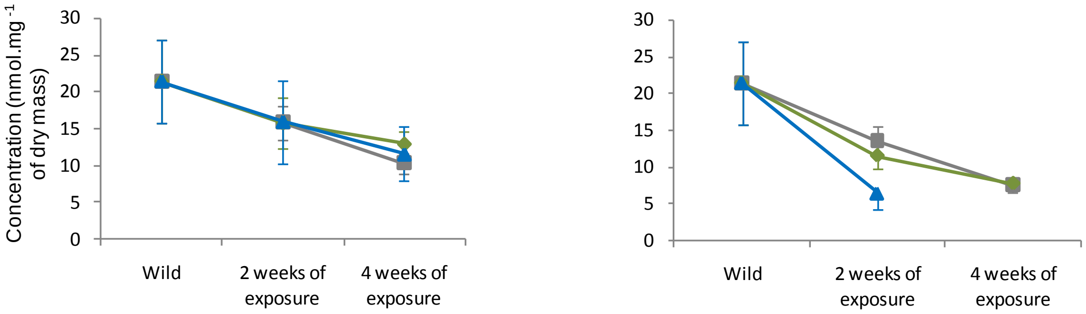


Fructose

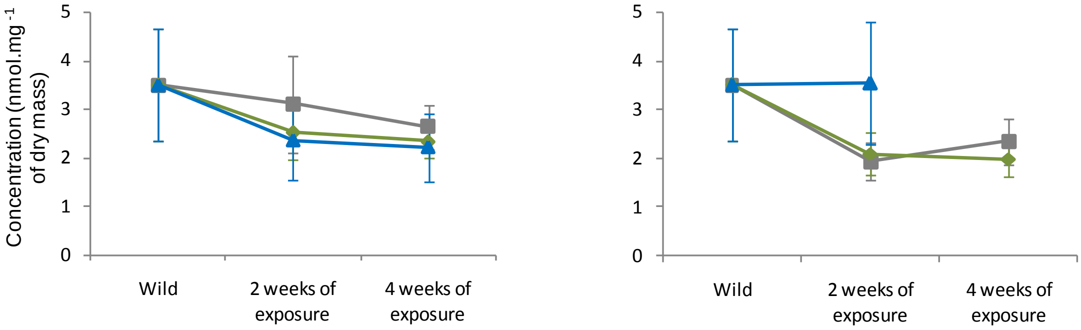


Galactose

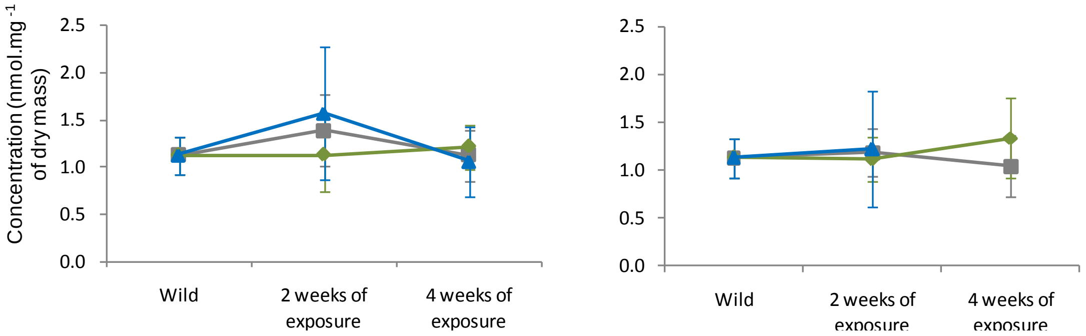
Gluconolactone



Glucose



Glucose 6 phosphate



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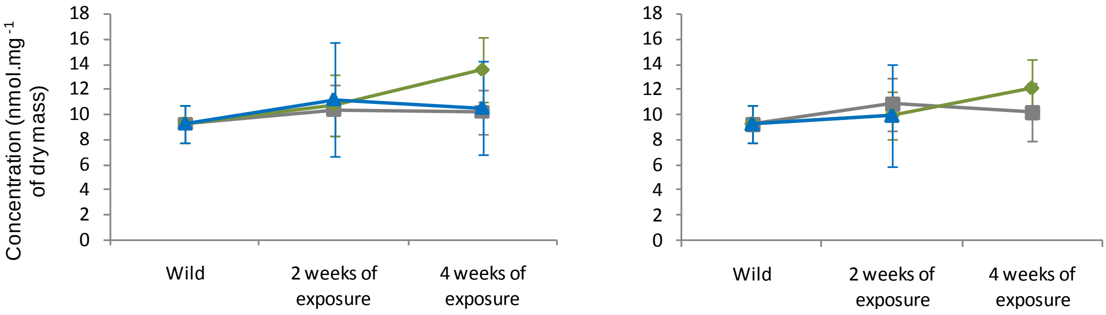
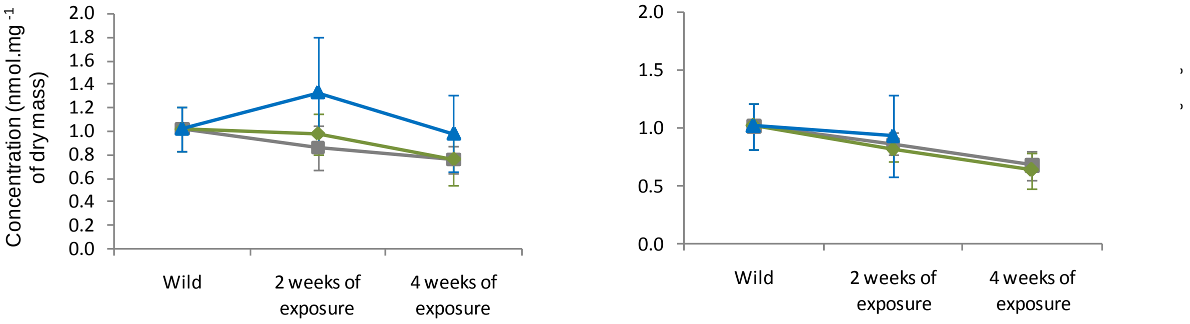
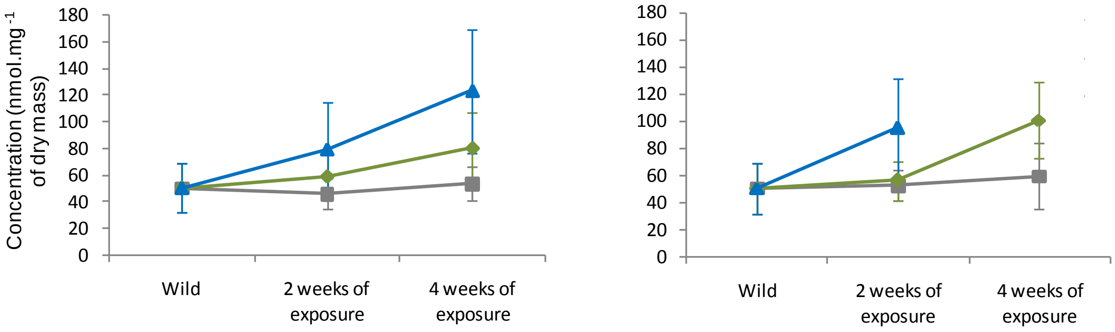



Glycerol
Concentration (nmol.mg -1 of dry mass)
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
Wild
2 weeks of exposure
4 weeks of exposure
2.0
1.5
1.0
0.5
0.0
Wild
2 weeks of exposure
4 weeks of exposure


Glycine
Concentration (nmol.mg -1 of dry mass)
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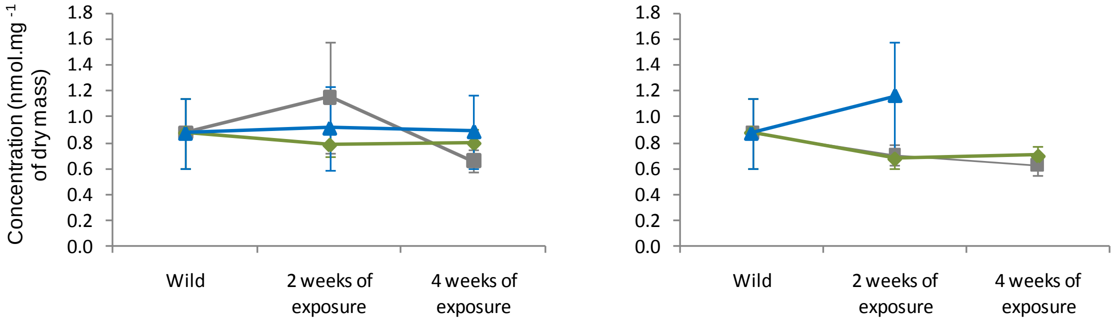
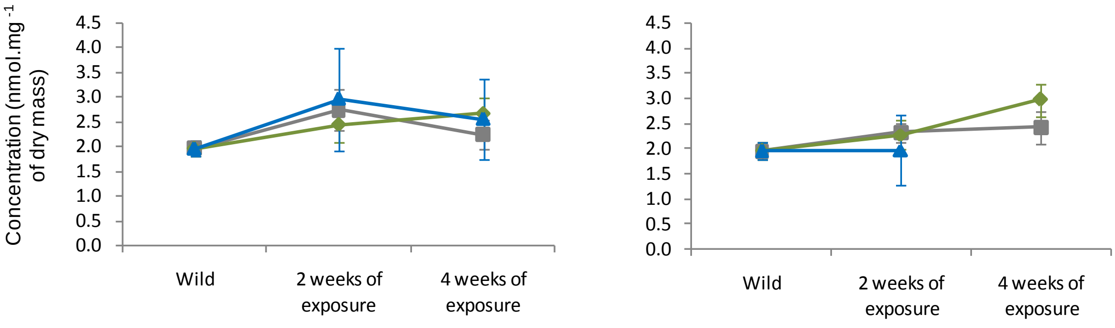
160
140
120
100
80
60
40
20
0
Wild
2 weeks of exposure
4 weeks of exposure
180
160
140
120
100
80
60
40
20
0
Wild
2 weeks of exposure
4 weeks of exposure


Inositol
Concentration (nmol.mg -1 of dry mass)
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
Wild
2 weeks of exposure
4 weeks of exposure
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
Wild
2 weeks of exposure
4 weeks of exposure


Isoleucine
Concentration (nmol.mg -1 of dry mass)
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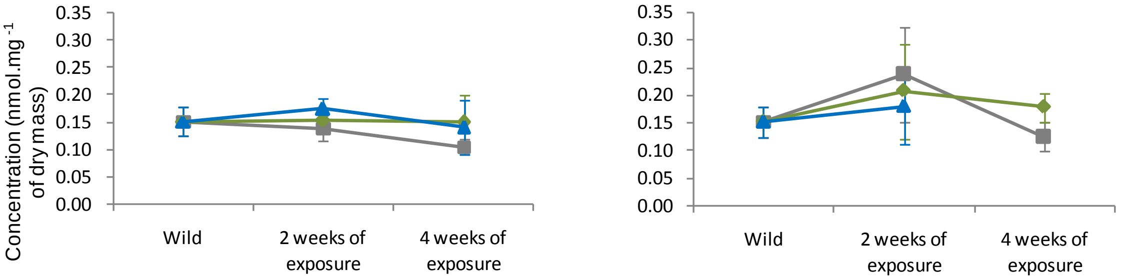
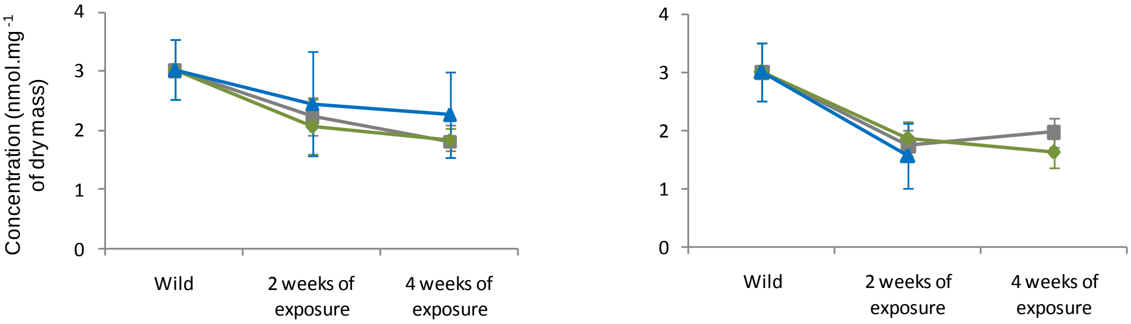
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
Wild
2 weeks of exposure
4 weeks of exposure
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
Wild
2 weeks of exposure
4 weeks of exposure

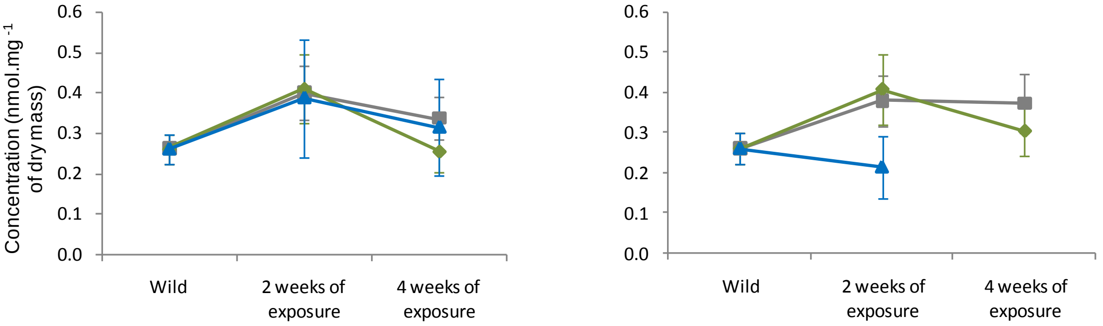
Mannitol



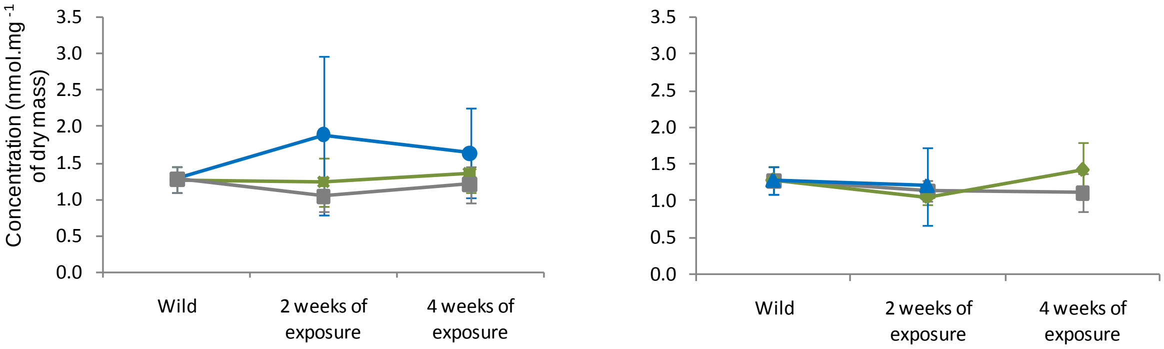
Mannose

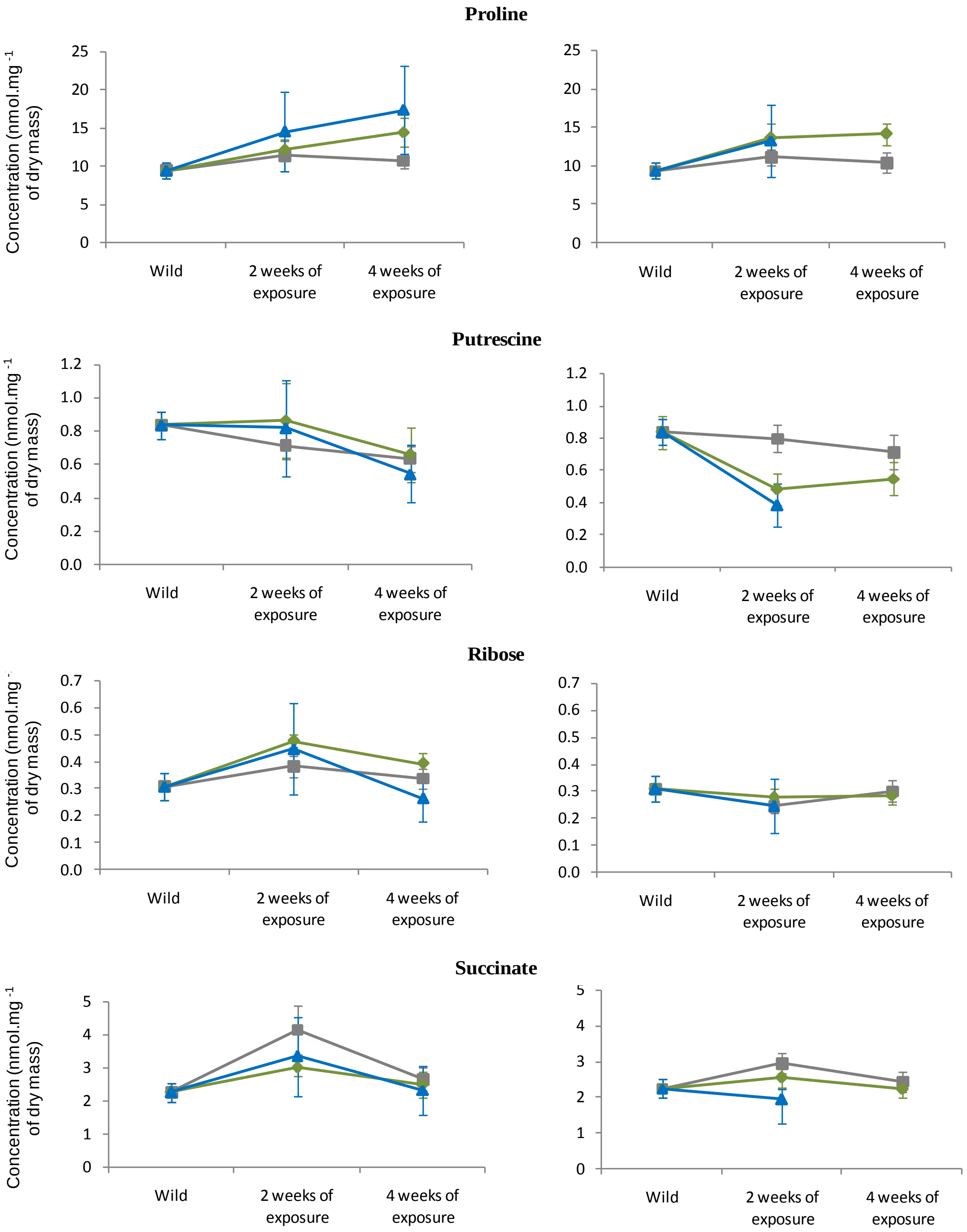


Ornithine

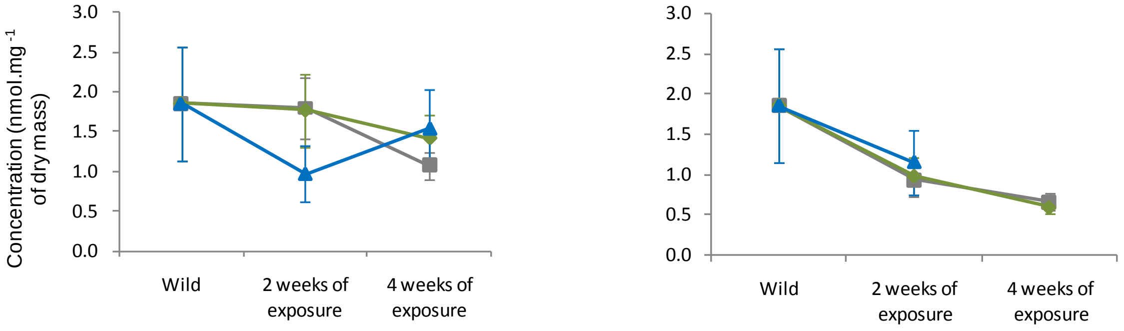


Pipecolate

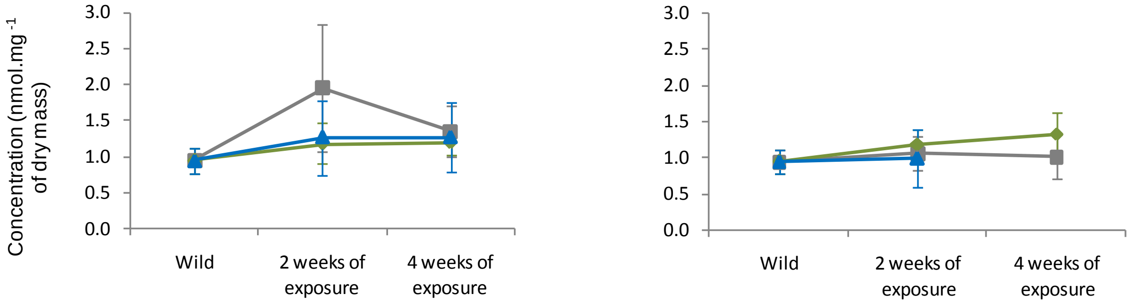



Proline
Concentration (nmol.mg -1 of dry mass)
0
5
10
15
20
25
Wild
2 weeks of exposure
4 weeks of exposure
Putrescine
Concentration (nmol.mg -1 of dry mass)
0.0
0.2
0.4
0.6
0.8
1.0
1.2
Wild
2 weeks of exposure
4 weeks of exposure
Ribose
Concentration (nmol.mg -1 of dry mass)
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
Wild
2 weeks of exposure
4 weeks of exposure
Succinate
Concentration (nmol.mg -1 of dry mass)
0
1
2
3
4
5
Wild
2 weeks of exposure
4 weeks of exposure

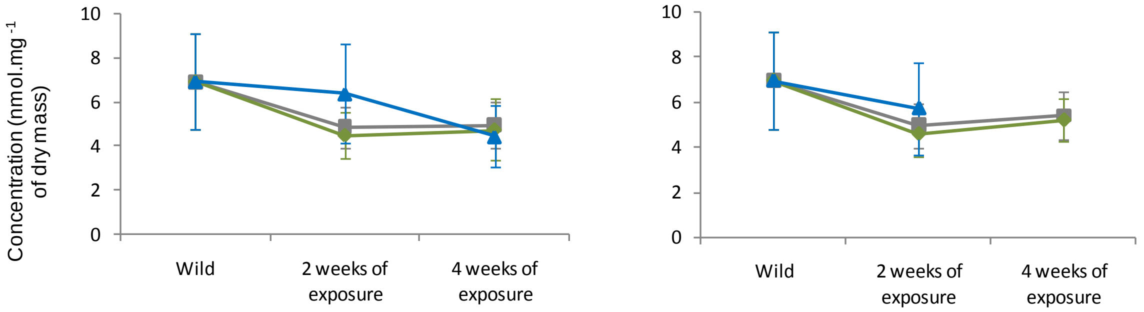
Trehalose



Erythritol



Valine



ESM 2. Molecular analysis supplement.

Sequence analysis

All insect species deposited in the GenBank database including HSC70 and HSP70 amino acid sequences were selected. The amino acid sequence of one HSP70 protein from *Microdera dzhungarica* (Accession number, JF421286), and of one HSC70 protein from *Copris tripartitus* (AN, ABP97091) were included in the phylogenetic analysis. After ClustalW alignment, sequences too short to provide informative phylogenetic data for tree-building purposes were eliminated, leading to a final dataset of 38 sequences. Maximum likelihood method (PHYML, www.atgc-montpellier.fr/phyml/; Guindon & Gascuel 2003) was applied using the LG model fixed by a Protest analysis to reconstruct phylogenetic relationships (Protest Server, darwin.uvigo.es/software/prottest_server.html; Abascal et al. 2005). Support for the topology was assessed by 100 bootstrap replicates.

Relationships between insect HSC70 and HSP70

Two sequence groups, supported by high bootstrap percentage values, are depicted in the ML phylogenetic tree (Fig. 1). The first group, which included the sequence generated in our study, corresponded to the constitutively expressed 70 kDa HSP; 20 of these sequences were clearly identified as HSC70. The second group corresponded to heat inducible forms; 16 of these sequences were identified as HSP70 (Fig. 1).

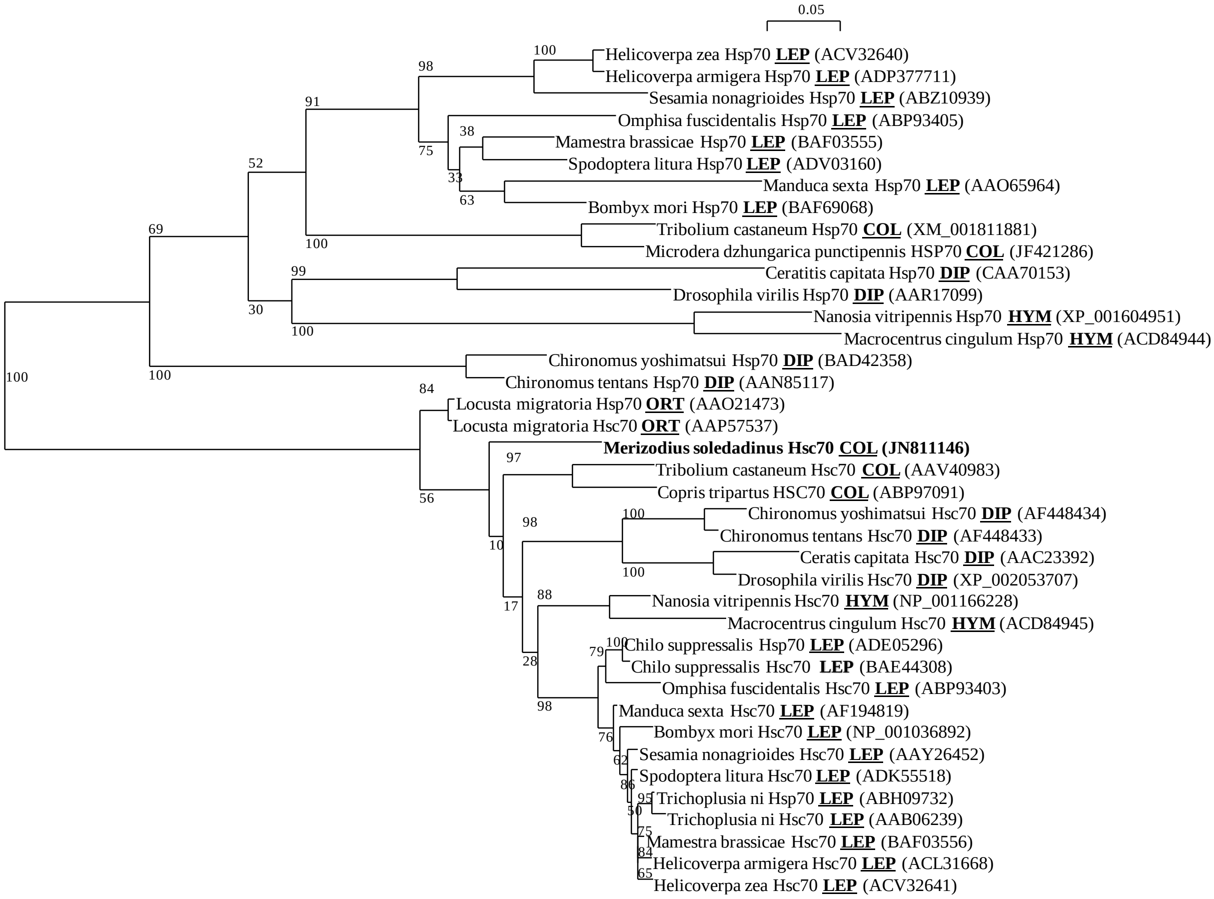


Fig. 1. Phylogenetic tree showing the relationships among HSC70 nucleotide sequences in insects. Thirty eight sequences of HSC70 and HSP70 from 19 insects were analyzed through maximum likelyhoot method under a LG model. The sequences clearly assigned to inducible (HSP70) or constitutive (HSC70) forms through expression analyses are annotated on the tree. The accession number of the nucleotide sequence is provided after each species name, and the sequences analyzed in this article are in bold font. Three letters codes indicate the order name of the corresponding insect (COL: Coleoptera; LEP: Lepidoptera; DIP: Diptera; HYM: Hymenoptera; ORT: Orthoptera). The values indicated on the branches correspond to bootstrap percentages (BP). According to this tree, there is a functional grouping of the sequences identified as HSP70 and HSC70.

3.3. Consequences of diet on energy budgeting in *Merizodus soledadinus* (ARTICLE VI)

Considering the vulnerability of native prey communities to the predation pressure by *M. soledadinus*, the durable success of this invasive insect at the Kerguelen Islands implies the ability of residents to withstand alterations of food conditions. By combining multiple approaches, the following article focuses on the outcomes of variation in availability and quality of trophic resources in *M. soledadinus*. The study spans from the monitoring of body mass and survival of *M. soledadinus* during food deprivation, towards the consequences of diet on the management of energy stores such as sugars and fat. This work on trophic plasticity and its metabolic consequences aimed at improving our understanding of the durable success of *M. soledadinus* despite its negative feedback on the available food in areas where its presence resulted in the extinction of some native prey species. It also contributed towards delineating how this invasive predator can benefit from other invasive species, that can represent consistent prey sources, more resistant to predation pressures than native ones.

Laparie, Larvor *et al.* 2011, *Comparative Biochemistry and Physiology Part A* Article VI

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Starvation resistance and effects of diet on energy reserves in a predatory ground beetle (*Merizodus soledadinus*; Carabidae) invading the Kerguelen Islands

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ABSTRACT

The relationship between nutritional requirements and the availability or quality of food is a prime parameter in determining the geographical expansion of invasive insects. At the sub-Antarctic Kerguelen Islands, the invasive ground beetle *Merizodus soledadinus* becomes the main invertebrate predator when it colonizes new habitats, leading to the local extinction of native fly species. Such changes in the structure of prey communities may alter the energy management (storage and expenditure) of this predator. In this species, we monitored survival and body mass during food deprivation, in addition to evaluating the effects of two distinct diets (maggots versus enchytraeids) on the consumption and restoration of body reserves (sugars and triglycerides). We found that adults can starve for more than 60 days, and feed every 3.76 days on average when food is available. We recorded higher predation rates on maggots, associated with steeper body mass variations, compared to enchytraeids. Sugars and triglycerides were significantly consumed during food deprivation and restored after refeeding, but varied similarly among individuals supplied on the distinct diets. Other parameters may determine the food preferences observed, such as salt content in prey tissues, because *M. soledadinus* mainly feeds in hypersaline foreshore habitats, and may limit the consumption of osmotic conformers.

1. Introduction

Variation in food availability and quality is one of the major causes of stress in animals (Rion and Kawecki, 2007; McCue, 2010). Access to trophic resources is triggered by seasonal variations, the depletion of transient food resources, inactive states (such as diapause, quiescence, or chill coma), or developmental processes (such as nymphal stage in holometabolous insects) (Tauber et al., 1986; Brakefield et al., 2007; McCue, 2010). Biological invasions also dramatically alter the availability of resources by disrupting species richness, as well as the structure and interactions of trophic webs (Vitousek, 1990; Crooks, 2002). Invasive predators may directly alter their own food resources by reducing the diversity and abundance of native prey (Snyder and Evans, 2006; Kenis et al., 2009). How such predators deal with food stress, and manage reserves in relation to food availability, is thus a key parameter in their long-term success in colonized areas.

Several insect species have been introduced to the sub-Antarctic Islands over the past century, despite the unrivalled geographical remoteness and low human visitation of these islands (Frenot et al., 2005; Chown et al., 2008). At the Kerguelen Islands, six out of the 26 naturalized alien arthropods became invasive (Frenot et al., 2005). On this archipelago, as well as on most other sub-Antarctic islands, native invertebrate communities are dominated by decomposers and host few predators (Schermann-Legionnet et al., 2007; Lebouvier et al., 2011). Therefore, alien insects that belong to the predatory feeding guild, such as the ground beetle *Merizodus soledadinus* (Coleoptera, Carabidae), are likely to have major ecological impacts. This flightless insect is the only invasive predator within this archipelago (Frenot et al., 2005). Since its introduction in 1913, it has become widely spread in coastal habitats, benefiting from low inter-specific competition, and the vulnerability of native prey species, which have evolved under low predation pressure (Lebouvier et al., 2011). Previous studies demonstrated that *M. soledadinus* rapidly becomes a dominant insect at sites colonized, leading to the local extinction of several native species, namely the wingless flies *Anatalanta aptera* (Diptera, Sphaeroceridae) and *Calycopteryx moseleyi* (Diptera, Micropezidae) (Chevrier, 1996; Chevrier et al., 1997; Frenot et al., 2008; Lebouvier et al., 2011).

M. soledadinus is considered to be a voracious generalist predator, feeding on mites, springtails, small insects, or the larvae of

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Hydromedion sparsutum (Coleoptera, Perimylopidae), which is an endemic beetle from the sub-Antarctic island of South Georgia where *M. soledadinus* was also introduced (Ernsting, 1993; Todd, 1996, 1997; Brandjes et al., 1999; Convey et al., 2010). Maggots, and even large caterpillars of *Pringleophaga kerguelensis* (Lepidoptera, Tineidae), are also preyed on by *M. soledadinus* at the Kerguelen Islands (Lebouvier et al., 2011). The persistence of dense populations of this polyphagous carnivore in areas of the Kerguelen Islands, where native flies were driven extinct (Chevrier, 1996; Lebouvier et al., 2011), implies the occurrence of obligatory diet shifts (Laparie et al., 2010). However, the energetic balance between the costs of predation (i.e., foraging and handling time) and the benefits of feeding (i.e., sugar and fat inputs for maintenance, dispersal, reproduction, and storage) is probably prey-specific, so that changes in the structure of prey communities may impact energy storage and expenditure in *M. soledadinus*.

Adult *M. soledadinus* are active year-round at the Kerguelen Islands (Chevrier, 1996; Chevrier et al., 1997), and may face food stress before dispersing and breeding. The rapid spread of this invader (up to 3 km y^{-1}; Chevrier, 1996; Lebouvier et al., 2011) and the patchy distribution of prey communities may result periods of fasting (McCue, 2010) during dispersal events, particularly in winter. Moreover, the adult life span is likely between one and two years, with numerous females reproducing one year after emergence. Indeed, Ernsting (1993) found that, despite seasonal reproduction and peak offspring emergence during late summer, many females are gravid or have even laid eggs in early summer, and thus emerged during previous summer.

In the present work, we examined the starvation resistance of adult *M. soledadinus*, and the biochemical consequences of diet on their body stores during variations in food availability, to improve our knowledge about the factors that contribute to the success of this invasive insect. We used two worm-like and apodal prey species that coexist in decaying seaweeds, which is a habitat that is frequently invaded by *M. soledadinus* at the Kerguelen Islands. The prey comprised (i) maggots of *Fucellia maritima* (Diptera, Anthomyiidae), which is an alien fly very similar to *A. aptera* and *C. moseleyi* in size, habitat and diet during larval stages, and (ii) enchytraeids, which maintain high densities even in invaded habitats. We monitored survival and body mass indices of adult *M. soledadinus* during food deprivation, in addition to evaluating the effects of diet on the consumption and restoration of body reserves (sugars and triglycerides). We expected (i) high starvation resistance, which would be an important asset for success in altered communities and during winter or dispersal, (ii) higher predation rate on maggots, which is supported by the regression of native flies in nature, and (iii) better digestive performance with this prey type (i.e., quicker meeting of body requirements).

2. Material and methods

2.1. Study animals

Adult *M. soledadinus* were hand-collected in January 2010 at Port-aux-Français at the Kerguelen Islands (49° 21′ S, 70° 13′ E), from under stones in coastal habitats dominated by *Aceana magellanica* grass, up to 15 m away from strand lines of accumulated seaweeds. Batches of 100 individuals were then transferred to controlled conditions at 8 ± 1 °C (75 ± 5% R.H., dark conditions) in plastic boxes (11.5 × l8.5 × 5 cm, $N = 10$), and supplied with water and food *ad libitum* (either maggots of *F. maritima* or enchytraeids) for two days. This experimental food supply was necessary to synchronize the nutritional state of all collected ground beetles. These individuals were then food deprived for different durations, and refed with the same prey type (maggots of *F. maritima* or enchytraeids) to investigate changes in energy stores. Additional individuals fed with *F. maritima* were used to measure the predation rate, as well as the duration of survival and body mass loss during prolonged starvation.

2.2. Predation rate

The daily predation rate was measured using 39 adult *M. soledadinus* that were fed for two weeks with maggots of *F. maritima*, and maintained under the same controlled abiotic conditions. These adults were placed in separate Petri dishes, the bottom of which was covered with a disk of paper (9 cm diameter) saturated with freshwater. One larva of *F. maritima* was placed in each Petri dish at the beginning of the experiment. Then, for each Petri dish, we checked daily whether the larva was intact and alive, or partially or totally consumed. When a larva was attacked or consumed (at least partially) by an adult *M. soledadinus*, a new larva was offered to the insect and the disk of paper at the bottom of the Petri dish was changed.

2.3. Starvation resistance

2.3.1. Duration of survival and body mass loss

To assess the duration of survival and the change in body mass during starvation, adult *M. soledadinus* were randomly placed in separately labeled Petri dishes containing paper saturated with freshwater. Individuals were maintained under the described controlled abiotic conditions. The ground beetles were individually weighed before the beginning of the experiment using a Sartorius® microbalance accurate to 0.001 mg. In the first experiment, 28 adults were individually weighed on a regular basis (i.e., every three days) for 50 days (except some weighing sessions that were advanced, postponed, or cancelled due to field work during the experiment). For individuals that survived more than 50 days, the sole monitoring of survival was then conducted. In the second experiment, we focused on a finer scale, by evaluating the first three weeks of food deprivation, and weighing 50 adults individually every day.

2.3.2. Body mass during food deprivation and subsequent renutrition

To address the possible effect of diet on body mass recovery after fasting periods, individuals fed for two days with maggots of *F. maritima* ($N = 80$) or with enchytraeids ($N = 80$) were placed in separate Petri dishes. The ground beetles were then food deprived for two or 14 days, and afterwards refed for 12 h with the prey type that they initially received. The fresh mass of each individual was measured at T0, just before refeeding (T1), just after refeeding (T2), and one week after refeeding (T3). In individuals that fasted for 14 days, an intermediate measurement was performed between T2 and T3.

2.3.3. Energy budgeting during food deprivation and subsequent renutrition

Twenty groups of 10 individuals were placed in Petri dishes, and food deprived for two or 14 days. They were then refed with maggots of *F. maritima* or enchytraeids for 12 h. The ground beetles were sampled at the beginning of this experiment (T0), just before refeeding (two or 14 days of food deprivation, T1), just after refeeding (T2) and one week after refeeding (T3). Once sampled, insects were immediately weighed (fresh mass), then stored in 800 µl 70% ethanol at −20 °C until the metabolic assays. For each sampling event, hereafter referred to as factor "Date" (T0–T3), eight replicates of three individuals were prepared for triglyceride and sugar assays.

The concentrations of triglycerides, glucose, and trehalose were measured in the whole insect body. Samples were vacuum-dried (MiVac, Genevac Ltd., Ipswich, England) and homogenized in 900 µL of methanol–chloroform solution (2:1) for sugars ($N = 8$ replicates for each experimental condition) or in 1200 µL of methanol–chloroform solution (1:2) for triglycerides ($N = 8$ replicates for each experimental condition). Homogenization was performed using a bead-beating apparatus Retsch MM301 (tungsten beads, Retsch GmbH, Haan, Germany) at 30 agitations per second for 90 s. Then, for sugars, 600 μL of ultrapure water was added to each sample and vortexed. After centrifugation at 8000 *g* for 10 min, 1000 μL aliquots of the upper aqueous phase, which contained polar metabolites, were transferred to microtubes, vacuum-dried, and dissolved in 300 μL of ultrapure water before assays. For triglycerides, we adapted a protocol from Hervant and Renault (2002). After homogenization using bead-beating, the samples were placed for 12 h at −20 °C, then 240 μL of KCl solution (2 g L^{-1}) was added and the samples were incubated for 5 min at 40 °C. Eventually, 640 μL of the lower phase (chloroform + lipids), was transferred to microtubes and dried at 30 °C under a nitrogen stream. The residual lipidic droplet was finally dissolved in 400 μL of Triton X 100 solution (0.2%) and delipidated BSA (3%), then vortexed and incubated for 10 min at 60 °C before metabolic assays.

We used spectrophotometric assay kits produced by Megazyme™ (K-TREH, Megazyme International Ireland Ltd.) and Cayman™ (Triglyceride assay kit, Cayman Chemical Company, Ann Arbor, MI, USA) to measure glucose, trehalose, and triglycerides. We followed the manufacturer's instructions, and adapted the volumes proposed by Megazyme™ to adjust the dosage of the sugars in the microplates.

2.4. Statistical analyses

Generalized Linear Models (Poisson family) were performed to investigate the relationship between starvation resistance (duration in days) and initial body mass for each gender.

In the food deprivation/refeeding experiments, the date (T0–T3) was considered as a factor. The variation of fresh mass was monitored for each ground beetle, resulting in dependent variables (weighing sessions). Thus, we adjusted the linear mixed effects models (fixed: prey*date, random: individual) for both experiments (two and 14 days of initial food deprivation). The initial measure (T0) was excluded from the models, as we examined body mass as a function of initial body mass in each individual, resulting in 100% values and no variance for all insects at T0. The two models were used to perform two-way ANOVAS with prey, date, and their interaction as factors. Afterward, we used the contrasts method to perform pairwise comparisons among groups with an α threshold of 0.05.

For metabolic assays, individuals were killed at each experimental stage to measure energy stores. All measures were therefore independent, and analyzed using linear models. In both experiments, a model was adjusted for glucose, trehalose, and triglycerides, resulting in six models. The models were used to perform two-way ANOVAS with prey, date, and their interaction as factors. We then ran *post-hoc* Tukey tests to perform pairwise comparisons among the groups when the factors were significant in the ANOVAS.

The prerequisites of adequacy to the normality of the residuals were checked using Q–Q plots and Shapiro–Wilk tests with an α threshold of 0.05. All statistical procedures except time–mortality regressions, were conducted with *R*™ 2.13.0 statistical software (R Development Core Team, 2004). Survival data were expressed as lethal times for 50% and 90% of the samples (Lt_{50} and Lt_{90}, respectively). Time–mortality regressions and days to Lt_{50} and Lt_{90} were calculated using probit analyses. These statistical analyses were conducted using *Minitab*™ 13 statistical software (MINITAB Inc., State College, PA, USA). Means are given with standard error in the text.

3. Results

3.1. Predation rate

Adult *M. soledadinus* attacked maggots of *F. maritima* 3.67 ± 0.31 times on average during the 14-day experiment. It corresponded to a mean rate of 0.27 ± 0.023 predation events per day, or one attack every 3.76 days.

3.2. Starvation resistance

Starvation resistance was measured until death in 25 individuals (three out of the initial 28 individuals escaped after 50 days of the experiment, they were therefore excluded from the survival analysis). Based on this experiment, we found a maximal starvation resistance of 105 days under controlled conditions, with an Lt_{50} and Lt_{90} of 60.50 ± 0.72 and 87.29 ± 1.18 days, respectively. No individual died before 27 days of food deprivation, and approximately two thirds of individuals survived more than 50 days (Fig. 1).

The body mass over a 50-day food deprivation period followed a discontinuous pattern with two steps, whereby body mass loss was steeper during the first three days of the experiment than during the remaining 47 days (Fig. 1). The two periods each correspond to an average loss of 15% of the initial body mass, despite different durations.

To assess the benefits of being larger during food deprivation, we plotted starvation resistance against initial mass (Fig. 2), considering mass as a measure of body size (Jarosik, 1989; Hodar, 1996; Laparie et al., 2010). Gender was also taken into account despite the biased sex ratio found in the sample (18 males and 7 females). The associated generalized linear models resulted in a significant relationship between initial mass and starvation resistance in females only, with 54.05% of explained deviance (Fig. 2).

In the second experiment, we focused on the first three weeks of food deprivation, to visualize clearly the entry into food stress and the transition between the two stages of body mass loss. Consistent with the previous experiment, we observed steeper mass loss during early fasting, with the first three days accounting for almost 10% of mass loss (Fig. 3). The rate of body mass loss decreased after one day of food deprivation. Body mass decreased at a slower rate in this experiment than in the previous one.

3.3. Refeeding and effects of the supplied diet

Not all individuals gained mass after they were refed, indicating that not all of them consumed prey items (Table 1). Overall, the attack rate was higher on maggots of *F. maritima* than on enchytraeids, albeit the difference tended to be lower after two days than after 14 days of food deprivation. Body mass gain was higher with maggots than with enchytraeids, as it resulted more often in an overreach or at least gain of more than 5% of the initial body mass. Of note, 83% of the individuals refed with enchytraeids after two-day food deprivation overreached their initial body mass. However, only 67% regained 5% of their initial body mass, indicating that the mass loss resulting

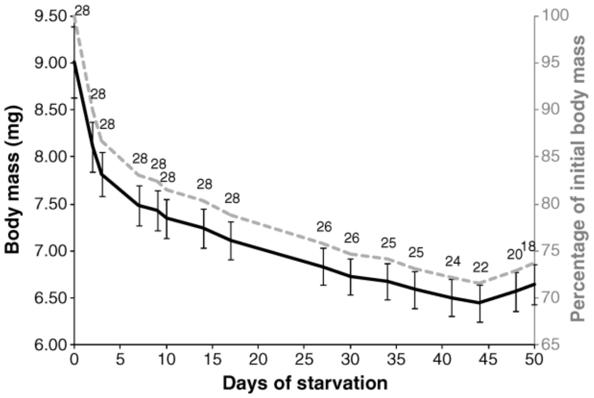

Fig. 1. Variation of fresh mass during a 50-day food deprivation period, averaged from 28 adults of *M. soledadinus* weighed individually. Error bars: standard error. Solid black line: body mass (mg); dashed grey line: percentage of initial mass. The number of surviving individuals at each weighing session is mentioned above the curves.

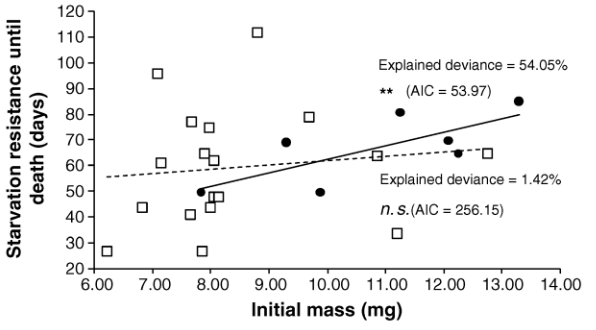

Fig. 2. Generalized Linear Models depicting the relationship between initial mass and starvation resistance in males and females starved until death (see Fig. 1). Open squares and dashed line: males; solid circles and solid line: females. Significance codes: <0.01 "**"; not significant "n. s.".

Table 1
Proportions of individuals which consumed prey when refed for 12 h (T2), after a two-day or a 14-day food deprivation. The proportions were computed from individual body mass comparisons before (T1) and after (T2) prey supply. Individuals which were lighter at T2 were considered as not refed.

Dataset	Subset	Maggots	Enchytraeids
14-day food deprivation	Refed	88.9% (*N* = 32)	58.3% (*N* = 21)
	Refed that recovered > 5% of initial mass	87.5% (*N* = 28)	47.6% (*N* = 10)
	Refed that overreached initial mass	81.3% (*N* = 26)	42.9% (*N* = 9)
Two-day food deprivation	Refed	74.4% (*N* = 29)	66.7% (*N* = 24)
	Refed that recovered > 5% of initial mass	100% (*N* = 29)	66.7% (*N* = 16)
	Refed that overreached initial mass	89.7% (*N* = 26)	83.3% (*N* = 20)

from two days of food stress was on average lower than 5% in these individuals (Table 2).

Further analyses were conducted only on individuals that actually consumed prey at T2. Whatever the duration of the initial food deprivation, we found no significant effect of the prey type on body mass. The effect of date (i.e., binary variations in food availability during the experiment) and the interaction between prey and date were significant (Table 2). These results demonstrate that, even if the overall mass of adult *M. soledadinus* (regardless of date) is not altered by the prey type, it evolved differentially over time according to the prey type, regardless of the duration of initial food deprivation.

Plots of body mass and the contrasts method showed a higher magnitude in body mass variation for ground beetles supplied with maggots, with an especially higher mass intake after refeeding (Fig. 4). As a result of these steeper variations, mass losses were higher in individuals supplied with maggots, but were offset by the higher mass intake that resulted from eating this prey.

3.4. Energy stores

The same experimental design was used to measure glucose, trehalose and triglycerides. Trehalose (except for the 14-day food deprivation experiment) and glucose contents significantly changed among dates (Table 3). These compounds were altered neither by prey type nor by the interaction between the factors of prey and date, as opposed to body mass (see Table 2). In both experiments, the variation in triglyceride content among dates was the most significant (Table 3). The prey effect was significant only in individuals that initially fasted for two days, but its interaction with date was not significant.

We performed pairwise comparisons among groups when the effects of factors were significant (Figs. 5 and 6). Overall, the tests confirmed the low variation of triglycerides, glucose, and trehalose among dates, indicating a limited consumption of energy reserves for the durations of food stress investigated. When pooling two-day food deprived individuals, regardless of diet (non-significant prey effect), we also found that glucose significantly decreased after the second fasting episode (one week, following the 12 h refeeding period). Trehalose also significantly increased during refeeding in these individuals (Fig. 5).

Triglycerides significantly decreased during the 14-day food deprivation period (Fig. 6). After refeeding, triglycerides were significantly reconstituted, and finally consumed again during the second event of food deprivation (one week), to a level similar to that observed just before refeeding (T1). A significant effect of the supplied prey was found in individuals that fasted for two days; therefore, these individuals were not pooled for pairwise comparisons. However, the *post-hoc* Tukey procedure rejected diet-related differences within dates. This test demonstrated that extreme means differed between the two diets, but not within comparable dates, which was supported by the non significance of the associated interaction term.

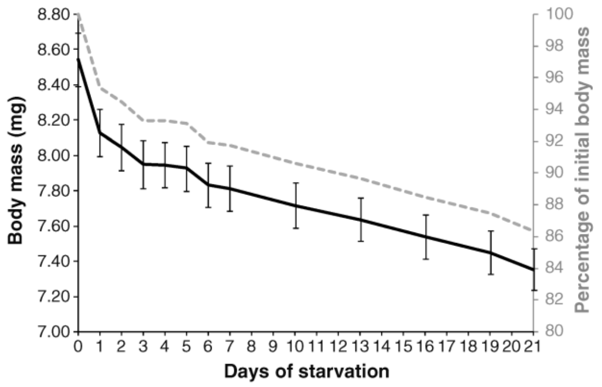

Fig. 3. Variation of fresh mass during a 21-day food deprivation period, averaged from 50 adults of *M. soledadinus* individually weighed. Error bars: standard error. Solid black line: body mass (mg); dashed grey line: percentage of initial mass.

4. Discussion

4.1. Starvation resistance in M. soledadinus

Starvation resistance is commonly considered as being size-dependent in different taxa of holometabolous insects (Łomnicki and Jasieński, 2000; Arnett and Gotelli, 2003; Reim et al., 2006). Intra-specific size variation is often related to the amount of reserves that individuals can store, or have already stored (Chippindale et al., 1996; Harshman et al., 1999, see review in Rion and Kawecki, 2007). Our results indicate that being larger significantly increased the resistance to food deprivation in female *M. soledadinus*, but not

Table 2
Results of the two two-way ANOVAS performed on the linear mixed effects models adjusted for individual body mass variation in each experiment (fixed: prey*date, random: individual). Significance codes: <0.001 "***"; not significant "*n. s.*".

Dataset	Effect	*df*	*F*	*p*
14-Day food deprivation	Prey	1	0.59	*n. s.*
	Date	3	84.34	***
	Prey:Date	3	15.19	***
Two-day food deprivation	Prey	1	0.79	*n. s.*
	Date	2	344.40	***
	Prey:Date	2	14.38	***

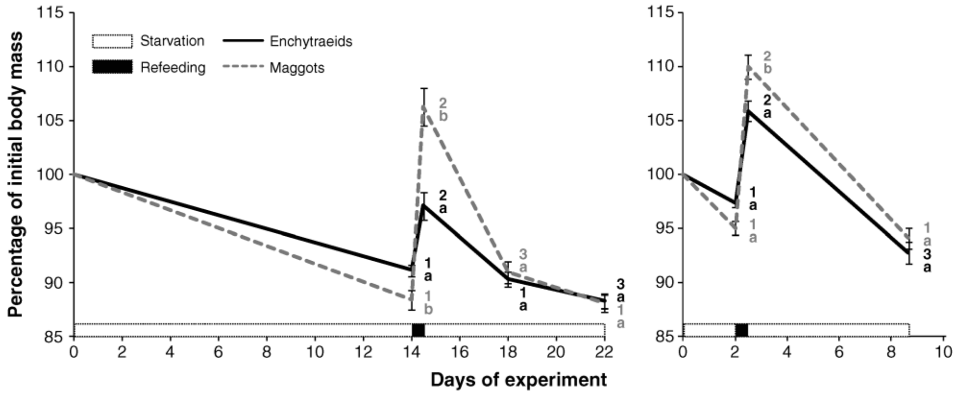

Fig. 4. Variation of body mass during food deprivation and subsequent refeeding in adult *M. soledadinus* supplied with two distinct prey types. Each ground beetle was weighed individually throughout the experiment; allowing the change in body mass to be computed as a function of initial body mass. Only individuals that actually ate between dates T1 and T2 were assessed (see Table 1). Left panel: 14-day initial food deprivation; right panel: two-day initial food deprivation. Solid black line: individuals initially supplied and refed with enchytraeids; dashed grey line: individuals initially supplied and refed with maggots. Error bars: standard error. Significance codes for the contrasts method used for linear mixed effects models (α threshold = 0.05): different numbers indicate differences among dates and within one prey type; different letters indicate differences between prey types and within dates.

in males, although future research should validate these analyses with larger sample sizes. Hence, the scaling relationship of the metabolizing tissue (Glazier, 2005) may be sex-dependant in this species. Blanckenhorn (2000) stressed that some counterbalancing forces may keep organisms small, as achieving large body size includes potential costs, such as high energetic requirements for maintenance and prolonged larval stages. Here we showed that these forces might apply differentially to males and females of *M. soledadinus*, which is consistent with morphological analyses previously conducted in the same species (Laparie et al., 2010).

The two-step decrease in body mass of adult *M. soledadinus* during food deprivation was consistent with the predation rate of this species. The reduction in catabolic rate, which was suggested by the decline in body mass loss when food deprivation exceeded four days, probably contributed towards increasing the duration of survival by ground beetles. However, monitoring the respiration and the locomotor activity would be necessary to confirm a reduction in catabolic rate through physiological adjustments or behavioral strategies (see for example the "sit and wait" strategy in Hoffmann and Parsons, 1993; Hervant and Renault, 2002; Renault et al., 2003; Issartel et al., 2010; Lalouette et al., 2011).

Table 3
Results of the six two-way ANOVAS performed on the linear models adjusted for glucose, trehalose, and triglyceride changes in the two-day and 14-day food deprivation experiments. Significance codes: <0.001 "***"; <0.05 "*"; not significant "*n. s.*".

Dataset	Compound	Effect	*df*	*F*	*p*
14-Day food deprivation	Glucose	Prey	1	2.59	*n. s.*
		Date	3	2.87	*
		Prey:Date	3	1.10	*n. s.*
	Trehalose	Prey	1	0.37	*n. s.*
		Date	3	1.30	*n. s.*
		Prey:Date	3	2.08	*n. s.*
	Triglycerides	Prey	1	0.34	*n. s.*
		Date	3	16.51	***
		Prey:Date	3	1.58	*n. s.*
Two-day food deprivation	Glucose	Prey	1	1.36	*n. s.*
		Date	2	3.58	*
		Prey:Date	2	1.82	*n. s.*
	Trehalose	Prey	1	3.17	*n. s.*
		Date	2	3.23	*
		Prey:Date	2	0.43	*n. s.*
	Triglycerides	Prey	1	6.02	*
		Date	2	13.40	***
		Prey:Date	2	2.17	*n. s.*

4.2. Prey preference and changes of reserves depending on food availability

Adult *M. soledadinus* are generalist predators that can attack a wide diversity of invertebrate species (Ernsting, 1993; Todd, 1996, 1997; Brandjes et al., 1999; Convey et al., 2010; Lebouvier et al., 2011). However, it is widely recognized that generalist insect predators may select and avoid certain species (Richards, 1982; Houck, 1986). Prey detection and selection must be economical, and is achieved according to prey mobility, size, color, taste, odor, and profitability (Richards, 1982; Houck, 1986; Eubanks and Denno, 2000). It remains unknown whether adult *M. soledadinus* use these characteristics for prey selection; however, in the present work, we recorded higher attack rates in individuals supplied with maggots than in those supplied with enchytraeids, especially in the 14-day food deprivation experiment. This result suggests a pattern of prey preference in adult *M. soledadinus* that is consistent with previous studies that reported the high impact of this invasive predator on other Diptera species, especially native ones (Chevrier et al., 1997; Lebouvier et al., 2011). Both enchytraeids and maggots of *F. maritima* coexist in decaying seaweeds, and are presumably easy to catch because of their lack of defenses and extremely low locomotor and escape abilities. The different predation rates that were recorded here may result from two non-exclusive factors that remain to be addressed in future studies. First, a lower predation rate on enchytraeids may result from the avoidance of hypersaline diets because of osmoregulatory needs. Indeed, enchytraeids are osmotic conformers (Schone, 1971; Eguileor et al., 1989) and thus contain more salt than coexisting maggots from decaying seaweeds. Second, the preference for maggots may be a function of their profitability (ratio between benefits, or nutritional value, and foraging costs), and one attack event might result in a higher energetic income than for enchytraeids. Indeed, water content is generally low in insects, while holometabolous larvae have sufficient energy stores to survive metamorphosis, and the maggots of *F. maritima* are heavier than enchytraeids (*F. maritima* maggots: 2.4 ± 0.8 mg dry mass, $N = 16$; enchytraeids: 1.7 ± 0.6 mg dry mass, $N = 20$).

When only considering the ground beetles that ate when refed, prey type had no effect on overall body mass, regardless of the length of initial food deprivation. This result demonstrated that the body mass gain of (re)feeding strictly offsets mass loss during food stress for both diets. Interestingly, fasting ground beetles consumed more food upon refeeding when supplied with maggots than when supplied with enchytraeids. The associated high transitory increase in

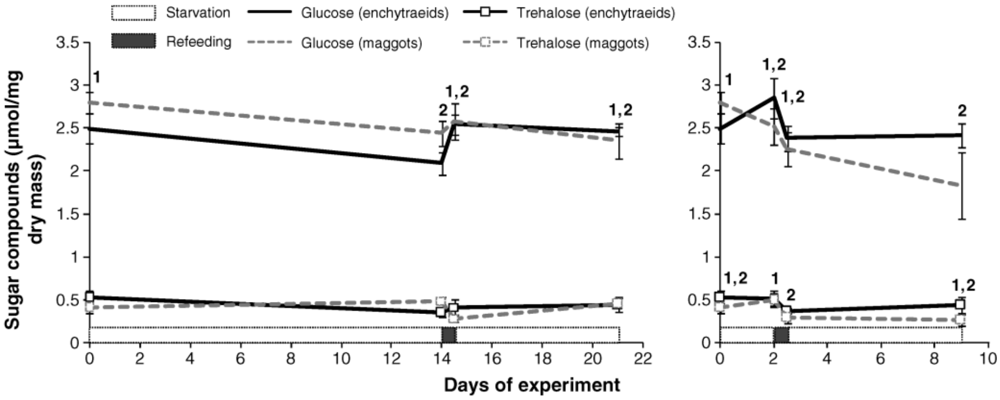

Fig. 5. Variation of glucose and trehalose contents during food deprivation and subsequent refeeding in adult *M. soledadinus* supplied with two different prey types. Left panel: 14-day initial food deprivation; right panel: two-day initial food deprivation. Solid black line: individuals initially supplied and refed with enchytraeids; dashed grey line: individuals initially supplied and refed with maggots. Open squares: trehalose; no symbols: glucose. Error bars: standard error. Significance codes for *post-hoc* Tukey procedures used for linear models (α threshold = 0.05): different numbers indicate differences among dates and within one prey type; different letters indicate differences between prey types and within dates. The pairwise comparisons were performed for significant factors only (see Table 3).

body mass of all individuals refed with maggots is of particular interest. Indeed, we observed physogastry (i.e., abdominal distention) in both males and females of *M. soledadinus* refed with maggots. The subsequent decrease in abdomen size and body mass over the next two days may result from an upregulation of diuresis (Maddrell, 1964), which is a loss of body water when body stores are restored, as shown by the synthesis of triglycerides.

As *M. soledadinus* is a true predator that can completely consume the prey individuals it attacks (Chevrier, 1996), the higher food intake associated with maggots may result from their higher dry mass, as compared to enchytraeids. However, both diets were supplied *ad libitum*, so that the total amount of food provided did not influence the amount of food swallowed. The predation rate and motivation of *M. soledadinus* to forage may be behavior-dependent, rather than being related to the quantity of ingested food, at least for the non-critical duration of food deprivation investigated here. One capture and refeeding event may be sufficient to temporarily lower the motivation of the predator, and end the search for another food item, whatever the size of the food item ingested.

Many authors have emphasized the relationship between endogenous reserves and starvation resistance in fruit flies (see review in Rion and Kawecki, 2007). We focused on triglycerides, glucose, and trehalose because fat is commonly the main energy reserve in insects (Arrese and Soulages, 2010), while carbohydrates can be catabolized quickly for dispersal, in addition to increased starvation resistance (Djawdan et al., 1998). Our results indicate the significant consumption of triglyceride stocks during food stress, as well as their significant reconstitution after refeeding. A similar pattern was found for carbohydrates, albeit it was less significant, suggesting that fat is the primary energy substrate of fasting *M. soledadinus*. These compounds varied even in the two-day food deprivation experiment, demonstrating that they are immediately mobilized during food shortage.

The management of the body reserves over the course of the experiment was similar in individuals supplied with distinct diets. The only diet-related difference was found in the two-day experiment when pooling dates, with individuals supplied with maggots exhibiting higher amounts of triglycerides (see Table 3). The higher body mass gain of ground beetles refed with maggots may thus involve the storage of other compounds. In particular, non-lipidic reserves like glycogen (Djawdan et al., 1998), proteins (Hervant and Renault, 2002), and free-amino acids, such as proline (Rankin and Burchsted, 1992; Gäde and Auerswald, 2002; Scaraffia and Wells, 2003), are

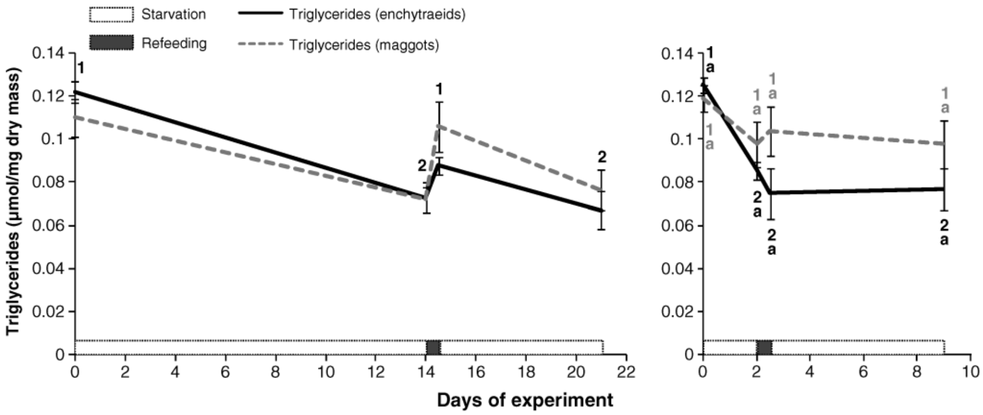

Fig. 6. Variation of triglyceride content during food deprivation and subsequent refeeding in adult *M. soledadinus* supplied with two different prey types. Left panel: 14-day initial food deprivation; right panel: two-day initial food deprivation. Solid black line: individuals initially supplied and refed with enchytraeids; dashed grey line: individuals initially supplied and refed with maggots. Error bars: standard error. Significance codes for *post-hoc* Tukey procedures used for linear models (α threshold = 0.05): different numbers indicate differences among dates and within one prey type; different letters indicate differences between prey types and within dates. The pairwise comparisons were performed for significant factors only (see Table 3).

significant energy substrates in invertebrates. However, the latter compounds may be viewed as "last chance" fuels during prolonged starvation because of the costs of protein breakdown (i.e., dispersal and muscle integrity; Hervant et al., 2001); hence, these compounds were not investigated during the moderate stress imposed in the present study.

Alternatively, maggots may have lower energetic richness (i.e., energetic tissues versus non-energetic tissues) than enchytraeids, and thus lead to steeper variations in mass with low consequences from an energetic perspective. This is unlikely, because maggots are heavier and must store large amounts of reserves to resist starvation associated with future metamorphosis and subsequent dispersal for reproduction, whereas enchytraeids have higher water content, thus reducing their energetic richness. Finally, the two-day and 14-day fasting periods both represent limited stresses, considering the high starvation resistance found in *M. soledadinus*. Longer periods of food deprivation after refeeding would have been necessary to tackle differences in reserve budgeting between the two diets. In addition, in these experiments aimed at assessing energy stores over time, we could not select individuals that actually fed, as insects could not be individually weighed throughout the experiments to check for mass gain.

5. Conclusions and perspectives

Our results suggest higher predation rates on maggots than on enchytraeids, which is supported by the local extinction of native flies after the predation of colonizing *M. soledadinus* in nature (Chevrier, 1996; Chevrier et al., 1997; Frenot et al., 2008; Lebouvier et al., 2011). However, the two diets produced only limited differences in energy budgeting. Biochemical assays were conducted for durations of food deprivation shorter than the maximal resistance found in this species. Hence, energetic differences between diets might only appear after critical starvation, or in storage compartments not investigated here. This finding helps towards understanding the persistence of large populations of the ground beetle in areas where native flies have been driven to extinction, while populations of enchytraeids remain extensive even in habitats colonized by *M. soledadinus*, and may provide a sufficient energy source. In addition to energy income, other factors may influence the predation rate between maggots and enchytraeids, namely the dietary avoidance of hypersaline food items (i.e., osmotic conformers) may help this species to withstand stress and ensure osmotic regulation.

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3.4. Are native species plastic enough to survive in their changing world? (ARTICLE VII)

Climate changes, habitat destruction and invasive species were identified as major threats to biodiversity worldwide by the Millennium Ecosystem Assessment (MA). The physiological perspective has much to offer to resolve and elucidate the impacts of these drivers and their interactions (Chown & Gaston 2008). Yet, the importance of understanding physiological capacities and plastic responses of species to these threats have long been underappreciated (Chown et al. 2010). At the Kerguelen Islands, the native wingless fly *Calycopteryx moseleyi* is thought to shift its ecological range towards secondary habitats and food resources in response to global changes (Vernon et al. 1998). The following manuscript addresses this hypothesis with special emphasis on the physiological phenotyping of this species between its contrasted primary and secondary niches at the Kerguelen Islands. This work aims at better understanding the scope that this species has for withstanding its changing world.

Laparie, Bical *et al. Submitted to Metabolomics* Article VII

Habitat phenotyping of two sub-Antarctic flies by metabolic fingerprinting: evidence for a species outside its home?

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Abstract

Metabolic fingerprinting can serve to elucidate plastic rearrangements of metabolic networks in organisms exposed to variable environmental conditions. Maintenance of organismal performance occurs by alterations in metabolic fluxes and pathways, resulting in habitat-specific metabolic signatures. Several insects distributed in the French sub-Antarctic Islands, including the wingless flies *Anatalanta aptera* and *Calycopteryx moseleyi* are exposed to hypersaline organic matter accumulations along littoral margins. However, *C. moseleyi* has long been considered restricted to Kerguelen cabbage which occurs from the foreshore, towards high mountain altitudes further inland. Therefore, high *C. moseleyi* densities identified in hypersaline seaweed are intriguing, and may involve osmoregulatory processes, such as the accumulation of osmoprotectants. In the present work, we examined quantitative metabotypes (metabolic phenotypes) among wild *C. moseleyi* individuals from seaweed and non-saline Kerguelen cabbages. Metabotypes from wild *A. aptera* individuals, the main competitor of *C. moseleyi* on seaweed, were also compared. Statistical procedures designed to emphasize inter-group differences indicated high similarities between *C. moseleyi* metabotypes from Kerguelen cabbage and seaweed, despite contrasted morphotypes, diets, and saline conditions. *Anatalanta aptera* exhibited higher glycerol, inositol, and other osmoprotectant concentrations that may assist in its enhanced performance under a hypersaline environment. Seaweed may represent a secondary (suboptimal) niche in *C. moseleyi*, promoted by a marked reduction in Kerguelen cabbage frequency subsequent to climate change, and herbivorous pressures caused by rabbit invasion on the islands.

Keywords

Compatible solute, gender, global changes, insect, Kerguelen Islands, osmoregulation, salinity, stress, survival.

Introduction

Phenotypic plasticity is defined as the expression of several phenotypes in response to different environmental conditions, with a single underlying genotype (West-Eberhard 1989). It is a central attribute that describes the range of spatial and temporal conditions an organism can withstand. Environment-induced phenotypes are observed at different organizational levels, including morphological (morphotypes), physiological (physiotypes), or metabolic (metabotypes) phenotypes (Nelson 1970; Gavaghan et al. 2000; Briegel 2003). Environmental metabolomics, a subdiscipline of metabolomics, is a nonbiased quantitative identification of all metabolites within an organism, and has been demonstrated as a convenient approach to delineate organism-environment interactions (Bundy et al. 2007; 2009). Indeed, many species inhabit a broad range of biotic and abiotic environments (Spicer and Gaston 1999; Gaston and Spicer 2001). In addition, environmental conditions are most often sub-optimal; therefore Darwinian fitness is never at its maximum *in natura* (Hoffmann and Parsons 1991).

The plastic rearrangement of metabolic networks, which results in the maintenance of near optimal organismal performance, establishes the existence of habitat-specific metabolic signatures (Szymanski et al. 2009). Variations in resource availability and quality alter metabolic signatures and key metabolites (Scherling et al. 2010). Adult morphology also reflects such dietary changes in holometabolous (Boggs and Freeman 2005; Laparie et al. 2010) and hemimetabolous insects (Hawlena et al. 2011). In addition, identical biomarker compound accumulations have been detected among distinct geographic sites sharing a similar environmental stressor(s) (Bundy et al. 2007).

Sub-Antarctic islands are dominated by decomposing communities of invertebrates feeding on massive amounts of organic matter produced along the foreshore by marine vertebrate colonies and seaweed accumulations (Crafford et al. 1986; Chown et al. 1998; Vernon et al. 1998; Convey 2001). These habitats are characterized by hypersaline conditions, due to tides, sea spray, and marine-related organic matter, which shape the geographical distribution of sub-Antarctic invertebrates (see earthworms in Frenot 1987). At the Kerguelen Islands, dense populations of *Anatalanta aptera* (Diptera, Sphaeroceridae) and *Calycopteryx moseleyi* (Diptera, Micropezidae) are distributed along littoral margins, in hypersaline habitats (Vernon et al. 1997). The two wingless fly species compete on organic matter accumulated under colonies of penguins (*e.g.* Macaroni penguins), or in decaying *Macrocystis pyrifera* seaweed (Tréhen and Vernon 1982; Tréhen et al. 1987).

During a 1984 expedition, Tréhen et al. (1987) noted surprise at large numbers of *C. moseleyi* not associated with populations of the Kerguelen cabbage (*Pringlea antiscorbutica*), as this plant has long been considered its main trophic resource and habitat. Jeannel (1964) earlier described *C. moseleyi* as subordinant to *P. antiscorbutica*, and reported that this fly did not shift towards alternate microhabitats despite a continued decrease in cabbage populations (both in distribution range and number of individuals). Kerguelen cabbages are distributed from littoral margins to further inland, and house large populations of *C. moseleyi*, namely in non-saline areas more than 90 or 140 m asl (see samples in Tréhen et al. 1987). Adults can be found at the axil of the leaves where rainwater is accumulated and salinity is null, or low compared to the soil. Tréhen et al. (1987) argued the shift to secondary niches they observed was facilitated by an extraordinary trophic plasticity in this fly, and was induced by external factors, *e.g.* habitat disturbance by native marine vertebrates (gentoo penguins, fur seals), and increased insular effects associated with small islands and islets. Vernon et al. (1998) added that rabbit invasion resulted in the quasi-extirpation of Kerguelen cabbage in several archipelago areas (see also Robin et al. 2011), forcing *C. moseleyi* individuals to seek other suitable habitats.

Little is known about the ecophysiolgy of the two native fly species, and the large *C. moseleyi* densities on hypersaline seaweed remain intriguing relative to its primary habitat. Hypersaline environments can impair osmoregulation by altering physiological parameters, such as ionic pressure of body fluids (Levin and Talley 2000). Saline tolerance involves compatible solutes (known as osmoprotectants) that contribute to protein denaturation and dehydration prevention by increasing body fluid osmolality (Somero and Yancey 1997; Majumder et al. 2010). The accumulation of polyols, soluble sugars, and free amino acids is a ubiquitous physiological response to high salinity levels in saline tolerant species (see Edwards 1982; Misra and Gupta 2005; Taji et al. 2006).

In the present study, metabolic fingerprinting, a global but non-exhaustive metabolite analysis, was used to examine quantitative metabotypes among wild individuals of *C. moseleyi* from non-saline *P. antiscorbutica* (Kerguelen cabbage) and *M. pyrifera* (seaweed), which were compared to *A. aptera* individuals collected from the same seaweed. We investigated if diet-induced metabotypes resulting from a habitat shift, *i.e.* Kerguelen cabbage to seaweed, were identifiable in wild *C. moseleyi* adults. Intraspecific differences among individuals were expected in samples from Kerguelen cabbage and seaweed. Assuming metabolic adjustments due to osmotic constraints under hypersaline conditions, we also hypothesized a level of interspecific similarities among insects sampled within decaying seaweed.

Material and methods

Insects

Anatalanta aptera and *C. moseleyi* adults were hand-collected in January 2010 at Pointe Suzanne, Kerguelen Islands (49° 26' S, 70° 26' E). *Anatalanta aptera* individuals ($n_{\text{males}} = 30$; $n_{\text{females}} = 30$) were sampled at the strand line level, in decaying seaweeds (*Macrocystis pyrifera* (L.) C. Ag. Laminariaceae), where the largest populations occur. *Calycopteryx moseleyi* individuals were sampled in the same decaying seaweeds ($n_{\text{males}} = 27$; $n_{\text{females}} = 27$), and from Kerguelen cabbage (*Pringlea antiscorbutica*, Brown Brassicaceae) leaves, 150 m from the littoral zone, and 50 m a.s.l. ($n_{\text{males}} = 30$; $n_{\text{females}} = 30$). Substrates were collected from both sites. All individuals were maintained on natural substrate at 4 °C (70 ± 5% R.H.) for four days, ensuring similar thermal regimes prior to obtaining measurements. Three individuals of the same gender, or two *C. moseleyi* sampled in seaweeds due to a larger body size, were pooled from each sample. Adult *C. moseleyi* collected in seaweeds were larger (36.2 ± 6.0 and 40.6 ± 12.9 mg of fresh mass for two male and female pools, respectively), compared with individuals collected from cabbage leaves (26.4 ± 5.5 and

25.3 ± 5.6 mg of fresh mass for three male and female pools, respectively). Individuals were placed in 1000 µL of 70° ethanol, and stored at -20 °C until analysis. A total of 58 samples were prepared; ten replicates of each *A. aptera* gender from seaweed, and *C. moseleyi* from Kerguelen cabbage, and nine *C. moseleyi* replicates from seaweed.

Metabolite Assays

- Separation and derivatization

Gas Chromatography/Mass Spectrometry (GC-MS) was used to measure non-structural carbohydrate concentration, and amino and organic acids from the whole insect body. The samples in ethanol were vacuum-dried (Speed Vac Concentrator, MiVac, Genevac Ltd., Ipswich, England), and 900 µL of methanol-chloroform (2:1) was subsequently added. Samples were homogenized using a bead-beating device (Retsch MM301, Retsch GbmH, Haan, Germany) at 30 Hz for 90 seconds; 600 µL of ultrapure water was subsequently added, and each sample was vortexed. Following centrifugation at 8000 g for 10 min at 4 °C, 270 µL aliquots of the upper aqueous phase, which contained polar metabolites, were transferred to microtubes and vacuum-dried. The polar phase aliquots were resuspended in 15 µL of 20 mg.mL^{-1} methoxyaminehydrochloride (Sigma-Aldrich, St. Louis, MO, USA) in pyridine prior to incubation under orbital shaking at 40 °C for 90 min. Following incubation, 15 µl of N-methyl-N-(trimethylsilyl) trifluoroacetamide (MSTFA; Sigma, #394866) was added, and derivatization was conducted at 40 °C for 30 min under agitation.

- Metabolite analysis

The GC-MS system was comprised of a CTC CombiPal autosampler (GERSTEL GmbH & Co.KG, Mülheim an der Ruhr, Germany), a Trace GC Ultra chromatograph, and a Trace DSQII quadrupole mass spectrometer (Thermo Fischer Scientific Inc, Waltham, MA, USA). The injector temperature was held at 250 °C. The oven temperature ranged from 70 to 147 °C at 9 °C.min^{-1}, from 147 to 158 °C at 0.5 °C.min^{-1}, from 158 to 310 °C at 5 °C.min^{-1}, and remained for 4 min at 310 °C. A 30 m fused silica column (TR5 MS, I.D. 25 mm, 95% dimethyl siloxane, 5% Phenyl Polysilphenylene-siloxane) was used, with helium as the carrier gas at a rate of 1 mL.min^{-1}. One µL of each sample was injected using the splitless mode (25:1). MS detection was achieved using electron impact. Ion source temperature was set to 250 °C, and the MS transfer line to 300 °C.

All samples were run under the SIM mode (electron energy: -70 eV), which ensures increased sensitivity relative to full scan analysis by focusing on target metabolites (Waller et al. 2007). Therefore, we only screened for the 59 pure reference compounds included in our spectral database. GC-MS peaks were accurately annotated using both mass spectra (two specific ions), and retention index specific to each compound. A quality control containing the 59 pure compounds at 200µM was run every 15 samples to verify instrument performance, set intervention limits, and basic instrument validation for metabolite profiling (Fiehn et al. 2008). Randomized sample sequences were established for sample injection, and each sequence was initiated with a quality control. In addition, our CTC CombiPal autosampler (GERSTEL GmbH & Co.KG, Mülheim an der Ruhr, Germany) enabled online derivatization and standardization of the preparation process. Each sample was automatically prepared during GC analysis of the preceding sample, which ensured the highest possible throughput of the system.

More importantly, auto-preparation resulted in equal derivatization duration for each compound prior to injection. Calibration curve samples for 59 pure reference compounds at 10, 20, 50, 100, 200, 500, 700 and 1000 µM concentrations were run. Chromatograms were deconvoluted using *XCalibur* v2.0.7, and metabolite levels were quantified using the quadratic calibration curves for each reference compound and concentration. Arabinose was used as the internal standard.

Statistical analyses

Metabolic differences among the six following classes of samples were evaluated: *C. moseleyi* from Kerguelen cabbage (males, females), *C. moseleyi* from seaweed (males, females), and *A. aptera* from seaweed (males, females). Among non-structural carbohydrate concentrations, and amino and organic acids, 38 compounds were identified; therefore multivariate methods were employed for data analysis. Compounds beyond detection limits were eliminated from the analysis. Compounds exceeding a 90% correlation with other compounds were discarded to avoid redundancy and an overestimate in differences among classes in multivariate analyses. Fourteen variables (*i.e.* compounds) were discarded, and the 24 remaining compounds significantly accounted for separation among the six classes. We performed stepwise forward variable selection based on the minimization of the Wilks' λ criterion, using the *R* package *kalR*. The Wilks' λ statistic is derived from the ratio of the intra-class inertia and total inertia. The statistic corresponds to the proportion of variation that is not explained by the grouping factor (*i.e.*, the six classes). The lower the Wilks' λ (values range from 0-1), the higher the separation of classes. A subset of 18 discriminant compounds (*i.e.* variables) was selected with this automated stepwise approach based on *F*-test decision among models upon inclusion of variables.

Class separation by the 18 discriminant variables was subsequently investigated in a Linear Discriminant Analysis (LDA). Following consideration of differences detected among the three populations, we conducted a MANOVA on individual *C. moseleyi* populations to eliminate species effects (in the absence of *A. aptera*), and test for metabolic differences between the species' two habitats compared here (non-saline Kerguelen cabbage or seaweed). All analyses were conducted with *R*™ 2.11.0 statistical software (R Development Core Team 2008).

Results

Fitting the discriminant model

The Wilks' λ criterion reached an asymptote through the stepwise procedure used to select the discriminant compounds (Fig. 1). Therefore, the selected set of variables was sufficient to optimally separate the individual classes.

Low habitat effect in Calycopteryx moseleyi

Despite a statistical design that maximized differences among classes, results indicated very similar metabolic signatures among all *C. moseleyi* individuals and habitats (Fig. 2). Between-class inertia was 30.81 times higher than within-class inertia along the first Linear Discriminant axis (LD1), corresponding to clear dimorphism between *A. aptera* and *C. moseleyi* (from both habitats). *Calycopteryx moseleyi* individuals were characterized by increased galactose, sorbitol, and leucine levels, but lower levels of most other compounds correlated with LD1, including inositol, ethanolamine, glycerol, tyrosine, proline, alanine, fumaric acid, and ornithine (see Fig. 3A for between- and within- correlations of variables to LD1).

The between-class inertia was 9.63 times higher than the within-class inertia along LD2, however the differences were notably lower along this axis than LD1 (Fig. 2). The relative proportion of correlations between variables and within-class inertia for this axis supported this result (Fig. 3A and Fig. 3B). This axis separated males and females of both species, regardless of habitat. In the three populations, males had on average more gluconic acid lactone, valine, threonine, phenylalanine, leucine, ornithine, and alanine relative to females (Fig. 2). Alanine was also responsible for a comparatively high proportion of within-class variation (Fig. 3B).

Testing for differences among habitats and genders in *C. moseleyi*, a MANOVA confirmed the LDA results (Table 1). Significant gender and, but to a lesser extent, habitat differences were observed for *C. moseleyi.* Sexual dimorphism was similar between both habitats, demonstrated by non-significance of the interaction term (Table 1).

We compared total concentration of the 30 compounds that were within detection limits among the three fly populations, including gender, and the interaction in an ANOVA. First, total concentration of all compounds was significantly higher in males ($P < 0.05$), without a significant interaction, demonstrating the sexual deviation was similar in all three populations. Most importantly, the *post-hoc* Tukey's Test ($\alpha = 0.05$) revealed the highest total compound concentrations were in *A. aptera* (173.42 ± 12.70 nmol.mg^{-1} of dry mass on average ± SE), and *C. moseleyi* from Kerguelen cabbage and seaweed did not significantly differ (119.84 ± 12.01 and 110.93 ± 16.17 nmol.mg^{-1} of dry mass, respectively).

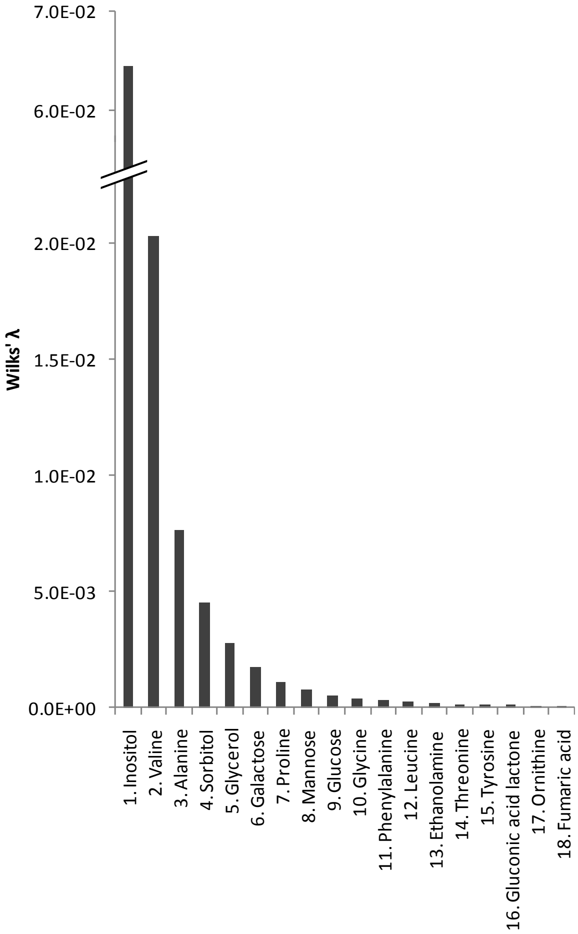

Fig. 1. Diminution of the Wilks' λ criterion resulting from the successive addition (1-18) of the discriminant variables in a stepwise procedure. The resolution of the discrimination reached an asymptote and would not be improved by adding more variables. Grouping factor considered: population by gender (six classes).

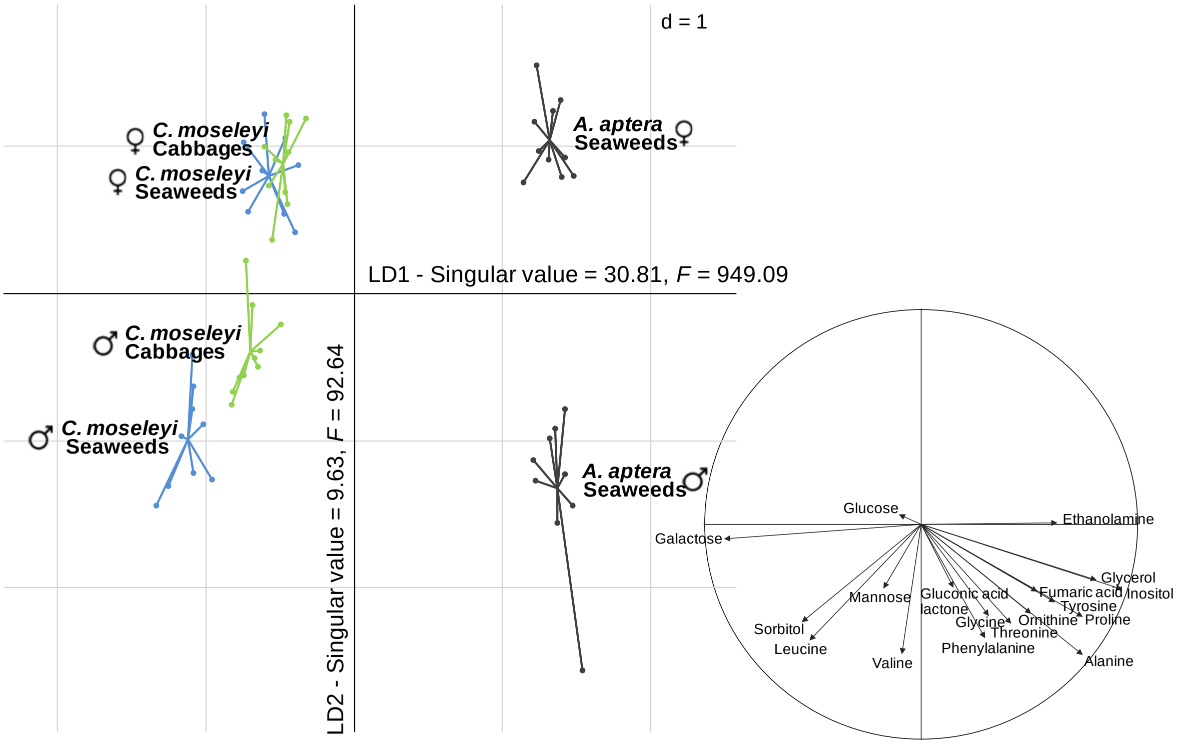

Fig. 2. Sample projection onto the first LDA discriminant plane. Grouping factor considered: population per gender (six classes). The lines link the samples to the centroid of their class. Blue: *C. moseleyi* collected from seaweeds; Green: *C. moseleyi* collected from Kerguelen cabbage; and Black: *A. aptera* collected from seaweeds. The correlations circle depicts the normed relation (from -1 to 1) between each compound and linear discriminant axes. The singular values are the ratio of between-class and within-class inertias.

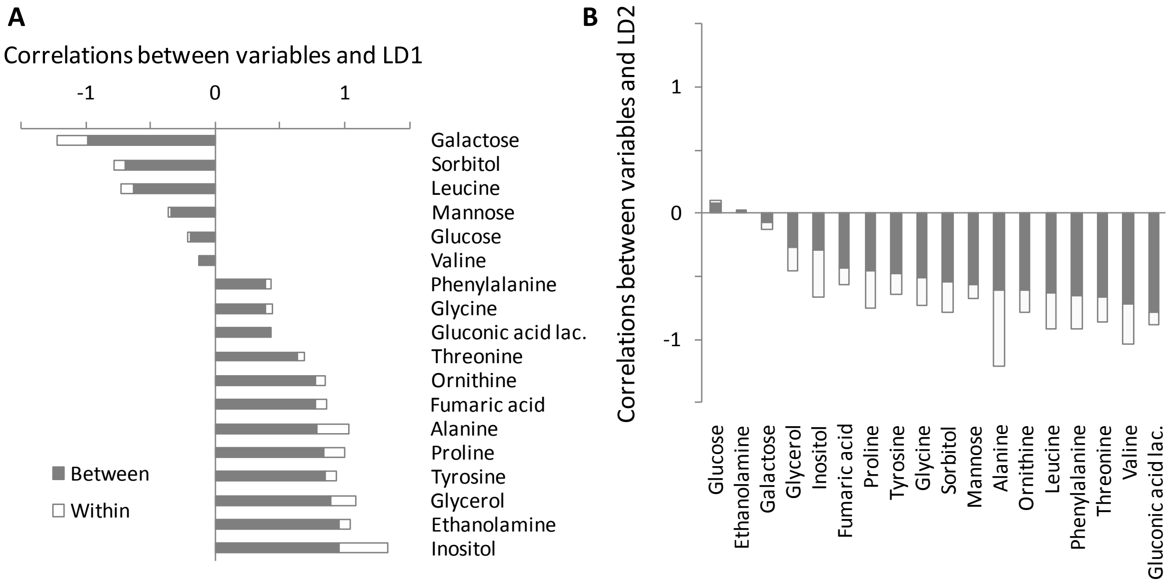

Fig. 3. Correlations between variables and within- or between-class inertia for LD1 (Fig. 3A) and LD2 (Fig. 3B). The variables are sorted depending on their between- correlation.

Table 1. Results of the MANOVA performed in *C. moseleyi* for the effects of Habitat, Gender, and their interaction. Significance codes: "*" $p < 10^{-2}$; "**" $p < 10^{-3}$; "***" $p < 10^{-4}$; "*n. s.*" not significant.

Dataset	Effect	F_{df}	Pillai * 10^2	*P*
C. moseleyi	Habitat	4.65_1	4.9	*
N = 38 (114 individuals)	Gender	21.79_1	6.6	***
	Habitat:Gender	1.82_1	65.80	*n. s.*

Discussion

Does the metabolic signature of Calycopteryx moseleyi *reflect its habitat and diet?*

A metabolome can be viewed as a phenotype-based description of a species niche, analogous to classical ecomorphological parameters (Wiens 1989). Compared to *A. aptera, C. moseleyi* individuals were characterized by increased leucine and sugar (galactose and mannose) levels. *Pringlea antiscorbutica* leaves, where *C. moseleyi* adults feed, contain high leucine-derived glucosinolate (n-butyl glucosinolate; Barillari et al. 2005) and soluble sugar levels (Aubert et al. 1999). In addition, flavonol glycosides (comprised of D-galactose or D-glucose sugar-residues), and agar (a galactose based polymer) are present in *P. antiscorbutica* leaves (Nielsen et al. 1993) and *Macrocystis pyrifera* (Percival 1979), respectively. On seaweed, where both species co-exist, *A. aptera* exhibited notably lower galactose levels relative to *C. moseleyi*. This compound may be an important characteristic of the energetic metabolism of *C. moseleyi*, regardless of the broad trophic plasticity in the species (Tréhen et al. 1987).

Contrasting morphotypes among *C. moseleyi* adult individuals sampled from seaweed and Kerguelen cabbage were observed. Consistent with these results, distinct morphotypes have been observed along salinity gradients in adult *Ochlerotatus taeniorhynchus* (Diptera, Culicidae) and *Chironomus salinarius* (Diptera, Chironomidae), likely the result of a longer period of development under increased saline conditions (Clark et al. 2004; Kefford et al. 2006; Cartier et al. 2011). Despite distinct morphotypes, and statistical procedures designed to emphasize inter-group differences, marked similarities among *C. moseleyi* metabotypes were detected from Kerguelen cabbage and seaweed. Congruent with the literature (Hines et al. 2007a; 2007b), increased gender compared to habitat differences were observed, even given the contrasted diet and saline conditions. Therefore, gender was more easily distinguished according to metabolic fingerprint than habitat. Females exhibited lower levels of amino acids, possibly due to lower amino acid requirements for vitellogenin synthesis (Hagedorn and Kunkel 1979). Interestingly, sorbitol accumulated in male *C. moseleyi*, but likely provided no osmoprotectant role, as it accumulated in individuals from both habitats. Overall, similar *C. moseleyi* metabotypes, regardless of habitat, emphasized the absence of osmoprotectant accumulation in individuals from seaweed.

Adult Calycopteryx moseleyi *may perform better in non-saline habitats*

In another study involving adults of both fly species from the same three populations, we could not sample *C. moseleyi* adult individuals experimentally exposed for two weeks at 35‰ salinity (see similar methods used in Pétillon et al. 2011) due to extreme mortality (100 and 82% mortality in *C. moseleyi* adults from seaweed and Kerguelen cabbage, respectively; *n* = 170). However, most *A. aptera* adult individuals from seaweed survived, and we proceeded with sample preparation. Therefore, *C. moseleyi* may elicit specific biological adjustments rather than physiological plasticity under saline conditions in nature. For example, Jury et al. (1994) reported behavioral avoidance of low salinity conditions in the lobster *Homarus americanus* (Decapoda, Nephropidae), (*i.e.*, stress level avoidance). We inferred survivorship from an experiment that prevented behavioral avoidance, which could explain the extreme mortality observed, even in individuals sampled from seaweed. On cabbages, adult *C. moseleyi* are found at leaf axils where rainfall accumulates (Tréhen et al. 1987). On some cabbages near littoral margins, and under spray influence, frequent washing by rainfall may locally reduce the salinity onto leaf axils. On seaweed, where salinity can reach higher values because of tide and evaporation, behavioral avoidance may require higher locomotor activity. However, adult *C. moseleyi* has been described as a motionless species with low metabolic activity (Verrall 1879; Jeannel 1940), whereas *A. aptera* is generally known as a highly mobile fly (Tréhen et al. 1987). Specific locomotor behaviors are consistent with the higher intermediary metabolite concentrations measured in adult *A. aptera*. In addition, behavioral avoidance of stressful saline conditions is not relevant in *C. moseleyi* larval instars. Maggots generally exhibit low dispersal power, which may underlie different modes of resistance among developmental stages.

Anatalanta aptera *may be physiologically advantaged in saline habitats*

Anatalanta aptera, a keystone species in recycling organic matter (Vernon 1981; Tréhen et al. 1987; Chevrier et al. 1997), was also observed in large densities on seaweed. Higher concentrations of several free amino acids including alanine, proline, and ornithine were detected, but also polyols *i.e.* inositol and glycerol. We previously isolated high proline and alanine levels from laboratory-reared *A. aptera* adults collected from Kerguelen and Crozet islands (Laparie M. & Renault D., unpublished data). These amino acids likely represent a metabolic signature in the species, regardless of sampling habitat type. Proline and ornithine are interrelated, and high proline levels are typically associated with metabolic and locomotor activities in insect species (Auerswald and Gäde 1999; Renault et al. 2006; Lalouette et al. 2007); proline is converted to alanine to fuel muscle cells (Gäde and Auerswald 2002; Scaraffia and Wells 2003). These compounds can also serve as osmoprotectants, and high concentrations may increase external ionic pressure resistance. Inositol and glycerol accumulation, and more generally polyols, also represent typical plastic responses in plants and arthropods under varied saline conditions (Patrick and Bradley 2000; Lee et al. 2008), and these molecules are considered major osmoprotectants (see for example Edwards 1982; Taji et al. 2006; Majumder et al. 2010). Even if hemolymph osmolarity resulted from a balance among different physiological processes, nearly all compounds were compatible osmolytes, and could consequently contribute to increasing body fluid osmolarity. Therefore, the higher total osmolytes concentrations in *A. aptera* compared with *C. moseleyi* (which, notably exhibited similar concentrations in all habitats) supports the hypothesis that *A. aptera* possesses a physiological advantage under hypersaline conditions.

We sampled *A. aptera* on seaweed, where the most abundant populations were found. However, the species is distributed in a wide range of other habitats along the salinity gradient, from the foreshore to further inland, and feeds on patchy vertebrate carrion (up to 700 meters asl; Vernon and Vannier 1996). Its tolerance to the magnitude of salinity change is likely due to physiological plasticity. Genetic metabolome specialization to every salinity modality is implausible; as it would require numerous segregated/isolated populations along the salinity gradient, and several distinct physiological tolerance specializations. In addition, individuals of this species exhibit high locomotor activity (de Blignières and Tréhen 1982; Tréhen et al. 1987; Chown et al. 2006), and are long-lived (Vernon 1981; Lalouette et al. 2010), suggesting that inter-population migration is common, and that one individual can encounter a wide range of saline conditions during its life.

A species driven beyond its home by global changes, a conservation perspective

We failed to identify habitat-related differences in *C. moseleyi* metabolic signatures. *Calycopteryx moseleyi* metabotypes showed no evidence of a plastic response to salinity, even in an osmotically challenging environment, and similarities with the better performing competitor *A. aptera* were not observed. Results indicated *C. moseleyi* adults suffered increased hypersalinity effects relative to *A. aptera*, despite the species co-occurrence in seaweed. Further studies are necessary to assess saline physiological plasticity of *A. aptera* adults, however the species survives better under hypersaline conditions, and exhibited metabolic properties advantageous to a hypersaline environment. *Calycopteryx moseleyi* showed the absence of physiological adjustments for the harsh strand line habitat, where interspecific competition is also much higher than in the Kerguelen cabbage. Further studies should investigate this paradox, and examine if additional secondary metabolites or ecological responses assist *C. moseleyi* performance along littoral margins.

Kerguelen cabbage is heavily endangered by introduced rabbits, and now by climate changes (warming plus dramatic decrease of rainfalls; see Lebouvier et al. 2011; Robin et al. 2011), therefore extending the threat to all islands, including those that remain pristine from rabbit invasion. The rapid and continued decline of this emblematic plant is likely the primary factor to explain the occurrence of *C. moseleyi* in new habitats (Vernon et al. 1998), outside its ecological preference, and its primary niche. Although additional studies are required to complete the present work, namely to examine the effect of habitat on *C. moseleyi*'s fitness, to our knowledge this is the first experimental evidence supporting the former idea that *C. moseleyi* has been displaced from cabbages to new and suboptimal habitats. These findings are likely critical for conservation of the wingless fly *C. moseleyi* endemic to Kerguelen and Heard islands.

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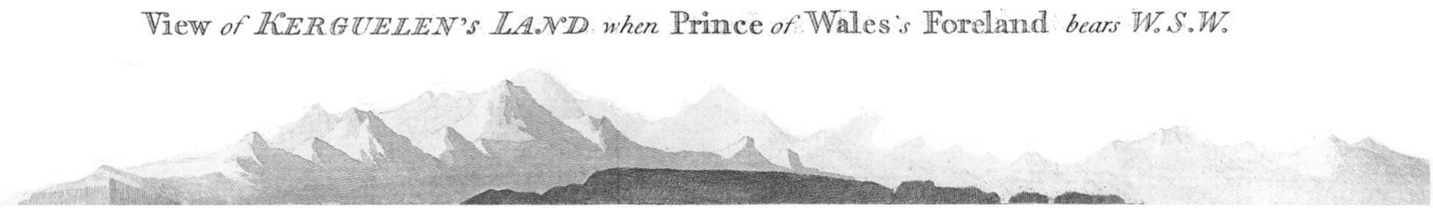
CHAPTER 4

GENERAL DISCUSSION



By William Bligh, Sailing Master of the *HMS Resolution* during the Captain James Cook's expedition in December 1776.

4.1. Fitting functional traits to newly colonized environments: a challenge for aliens

The success of invasive species is widely considered as depending on the adequacy between their life history traits and the environmental conditions (biotic and abiotic) in their new area (Thompson et al. 1995, Radford & Cousens 2000, Heger & Trepl 2003, Bass et al. 2006, Richardson & Pyšek 2006, Hayes & Barry 2008). This close relationship triggers the fulfillment of all stages of the invasion process, including survival during transport. Pre-adaptation of alien species – if their native range is similar to the invaded one (Facon et al. 2006) – together with post-introduction phenotypic plasticity and evolutionary processes (Dietz & Edwards 2006) are prime components of this match between species and environmental characteristics. As they progressively disturb the native ecosystem, even pre-adapted species shall meet changes of selection pressures. While adaptive changes may occur over the long term only, as a result of intergenerational processes, phenotypic plasticity is likely to assist the alien's performance as soon as it arrives in the new environment, by acting as a shield to protect from novel selection pressures (Roderick & Vernon 2009). Indeed, Schlichting (2004) stated that selection is blind to anything except the phenotype expressed in a particular environmental condition, regardless of the underlying mechanism for producing this phenotype. Consequently, species’ traits may suit the new conditions plastically and/or genetically, and their level of plasticity may also be selected through long-term processes (Schlichting 1986, West-Eberhard 1989, Crooks 2005, Dietz & Edwards 2006, Richards et al. 2006).

Functional traits are those that define the ecological roles of species, *i.e.*, how they interact with their environment and other resident species (Diaz & Cabido 2001). Functional traits range from body size and dispersal to metabolic rate or tolerance to environmental conditions, and strongly influence organismal performance (McGill et al. 2006). In this thesis, we characterized morphological and ecophysiological clues on the ability of both aliens, *C. vicina* and *M. soledadinus*, to match the variations in time and space of their newly colonized environments..

4.1.1. Morphological changes are multifactorial

Morphological traits can influence (directly or indirectly) individual performance and fitness, which in turn interact with the underlying genetic architecture to respond to selection (Arnold 1983, Kingsolver & Huey 2003). Morphology is partially under genetic control and may diverge in intraspecific clines (Garnier et al. 2005) that have received great attention by evolutionary biologists (Kingsolver & Huey 2003). Body size is also considered to be a key feature that can vary because of the effects of natural selection on the size-dependency of resource acquisition and mortality rates (Chown & Gaston 2010). Morphological traits also constitute sensitive tools for investigating patterns of differentiation that molecular markers may fail to detect (Nice & Shapiro 1999).

David et al. (2004) reported that the adaptiveness of phenotypic variation is generally considered both as evidence for and as a consequence of a history of selection. However, Debat & David (2001) reemphasized that some reaction norms cannot be presumed to be adaptive or not, as all biological processes result from a permanent interplay between internal constraints and natural selection (David et al. 2004). For instance, food shortage shall reduce metabolic activity and growth rate, but the resultant reduced adult body size can be more difficult to interpret (Stearns 1989, 1992, Charnov 1993, David et al. 2004). A set of different phenotypes produced by one genotype along an environmental parameter might also be the symptom of developmental sensitivity to this parameter rather than the expression of an adaptive phenotype (Debat & David 2001). However, an obligatory phenotype specifically expressed in a given location may be viewed as optimal as long as it is the only one a species can produce regarding the forces locally in action, be it the result of adaptation or simply developmental sensitivity. Reaction norms must then be distinguished from anarchic variations resulting from the disruption of canalization and developmental stability (*i.e.*, unconstrained developmental noise; Scheiner et al. 1991, David et al. 2004). They highlight the ability of organisms to survive and reproduce under a range of parameters by producing a set of consistent phenotypes triggered by environmental conditions, and are essential for understanding evolution in natural populations (Auld et al. 2010).

Here, we provided both geographical and allochronic patterns of morphological variations in two of the most successful invasive insects at the Kerguelen Islands. Considering the framework proposed by Arnold (1983), such variations through the invasion process may integrate possible changes in functional traits, performance and fitness that may have occurred over time and space because of evolutionary, plastic, or developmental processes. As discussed below, the resultant morphological changes probably result from a combination of the three processes. These changes are relevant markers of the success of the two invasive species considered, as they provide evidence of their ability to quickly fit and reproduce in a variety of new environmental conditions. In addition, the temporal and spatial scales at which the changes occurred are known, which is generally considered a crucial condition for inferring the respective magnitude of the evolutionary forces involved (Facon et al. 2006).

Merizodus soledadinus was first observed at the Kerguelen Islands by René Jeannel (Jeannel 1940). He compared native and alien individuals, reporting large fluctuations in the morphology of *M. soledadinus* within its native range, namely in size and shape of the pronotum. However, based on "very numerous specimens" (translated from Jeannel 1964) collected at the Kerguelen Islands (Port Couvreux), he noted that its introduction in this location formed a race with fixed morphological characters, of a great stability, without any interindividual variation (Jeannel 1962, 1964). More than 25 years after his expedition at the Kerguelen Islands, Jeannel published his curiosity about what had become of this alien species, discovered in ruins of human installations at Port Couvreux. He stressed that future naturalists should check the barracks of Port Couvreux and monitor what he named an ongoing natural experiment (Jeannel 1964). The success of *M. soledadinus* is now far beyond his original expectations, and Jeannel would be surprised for at least two reasons. First, he hypothesized the extinction of this alien ground beetle with the disappearance of the last ruins and barracks of Port Couvreux. He did not expect *M. soledadinus* to ultimately become one of the most invasive insects within the Kerguelen Islands, colonizing on its own several remote sites free of human activities, and rapidly becoming a dominant species (Chevrier et al. 1997; **ARTICLES I**, **III**, **IV** and **V**). Second, the populations formed through this invasive history differ in individual morphology, despite the strong morphological stability Jeannel observed in the original population in 1939 (**ARTICLES III** and **IV**). On the one hand, the appearance of new morphologies is difficult to reconcile with the Jeannel's finding, as he investigated the only founding population at the Kerguelen Islands, which presumably offered limited fuel for selection pressures on morphological traits. On the other hand, the fixed morphological characters that he reported are consistent with the significant discrimination among distinct populations, because the within-population heterogeneity is low enough to allow segregation on morphometrics. The question remains of what caused the geographical morphological pattern among populations of *M. soledadinus* at the Kerguelen Islands.

The morphological variation among populations may result from local conditions (either adaptive phenotypic plasticity or natural selection regarding available resources, or developmental sensitivity to a stressing parameter; **ARTICLE III**) and/or on the genotype of the colonists (hypothesized in **ARTICLE IV**). Contrasting with the low phenotypic variation observed by Jeannel (1962, 1964), and almost one century after introduction, Lalouette (2009) showed that the allelic richness within the Kerguelen Islands was the highest in the population from Port Couvreux. Altogether, these observations suggest that some external force may constrain phenotypic variation. Remarkably, in 15 other populations, the allelic richness decreased progressively with geographical distance from Port

Couvreux, the unique introduction point (Lalouette 2009). Hence, despite the presumably strong bottleneck that occurred during the introduction at the Kerguelen Islands, the geographical pattern observed in morphology is unlikely to result only from phenotypic plasticity. The founding population of Port Couvreux might thus have provided enough genetic variance for differences to appear among child populations. This hypothesis cannot be confirmed though, and should be considered with caution, as Lalouette (2009) investigated genetic differences using microsatellites, and noncoding DNA cannot be directly related to the phenotypic variations reported. Morphometrics corroborated genetics, as we found that the relative standard deviation for morphometrics was comparatively high in Port Couvreux (**ARTICLE III**). Nevertheless, while remaining high, it was not necessarily the highest among the five populations investigated (see females), suggesting that not only genetics, but other forces may shape the geographical pattern (**ARTICLE III**).

Because of metabolic trade-offs that constrain energy allocation to growth, maintenance and reproduction compartments (Moczek 1998, Karino et al. 2004), variation in food availability and quality are likely to alter adult morphology of insects (Boggs & Freeman 2005; **ARTICLE III**). Chown & Gaston (2010) reported that variation in nutrition have variable effects on growth rates and critical weight in insects (*i.e.*, the interval to cessation of growth), thereby influencing adult size. This is particularly consistent with the invasion of *M. soledadinus*, as its progression correlates with extinctions of native species that are abundant in pristine habitats (**ARTICLES I**, **III** and **VI**). The invasive success of a species must be considered over the long term rather than on an instantaneous snapshot (Strayer et al. 2006) because populations of invasive species often decline during the spread step, and ultimately fail for unknown reasons (see "boom and bust" phenomenon; Blackburn et al. 2011). The inability of some invasive species to quickly match the ongoing changes they cause to newly colonized environments is a good candidate for explaining such failures. The morphological differentiation of populations from habitats that differ in residence time (*i.e.*, mostly in the level of perturbation of prey communities) highlights the ability of *M. soledadinus* to withstand his own long-term negative feedback. It is a consistent fingerprint of the durable success of this carnivore at the Kerguelen Islands. Moreover, the strongly altered prey communities are now partly composed of species maintain large populatons in spite of predation by *M. soledadinus* (maggots of *F. maritima* and *C. vicina*, marine-related enchytraeids; **ARTICLE VI**), thereby suggesting that the negative feedback of *M. soledadinus* on its food may reach an asymptot over time. While being possibly suboptimal (because they are abundant during summer only) as compared to some native species that were quickly driven extinct, these less vulnerable species are actually preyed on by adult *M. soledadinus*, and apparently suffice to fulfill energy requirements (**ARTICLE VI**).

The reduction in allelic richness with increasing distance from the introduction point found by Lalouette (2009) may also reflect directional selection involving dispersal ability (**ARTICLE IV**). Many organisms show polymorphism in dispersal distance strategies (Roff 1986, Bonte et al. 2010), and the morphological variation may also be continuous rather than discrete (Hill et al. 1999a, Hill et al. 1999b, Merckx et al. 2006, Merckx & Van Dyck 2006). Here, this relationship with invasive front was investigated using residence time rather than geographical distance, as all child populations were not founded by individuals coming directly from Port Couvreux, but rather by individuals from other child populations in a stepping stone way. We highlighted a converse and partly predictable quantitative relationship between residence time and body size (**ARTICLE IV**). This result is consistent with theoretical view that larger insects have higher dispersal and invasive power, and may thus contribute towards expanding the distribution of invasive species within their new environments (Bowler and Benton 2005, Lawrence 1987, Anholt 1990, Mossakowski & Stier 1983). The validation of this fingerprint hypothesis requires further investigations of the relationship between morphometrics and invasive power. We already found that larger females may have a higher starvation resistance, possibly assisting survival during both active and passive dispersal events that imply fasting periods (see McCue 2010, Renault 2011; **ARTICLE VI**).

The morphological differentiation involved changes in traits associated with body size and reproduction *versus* changes in mouthparts associated with feeding. These changes may result from multiple factors. Larger body size may promote colonization of new sites by increasing performance during active or passive dispersal events (**ARTICLES III, IV** and **VI**). Eventually, traits associated with fecundity and feeding strategies regarding impoverishing prey communities may be prominent to successfully establish durable populations, while high energy requirements for large individuals or extended development may be disadvantageous (**ARTICLES III** and **IV**). In the light of the low variations observed in the original population of Port Couvreux by Jeannel in 1939, it is now of paramount importance to assess the morphological and genetic variations that exist in native populations of *M. soledadinus*. It will contribute towards better understanding the factors that stimulate and constrain phenotypic variations in the invaded areas, as well as deciphering the relative importance of phenotypic plasticity and directional selection in the patterns observed.

The blue blowfly *C. vicina*, that also successfully invaded the archipelago, has undergone wing size and shape alterations over time despite comparable thermal conditions over the periods compared (**ARTICLE II**). At Port-aux-Français, plastic effects regarding dietary conditions are unlikely to be the driving factor of the changes recorded. This saprophagous fly feeds on organic matter that have always reached high abundances in this site during the period considered (arrival of numerous elephant seals every summer, abundance of rabbits, waste disposal at the research station, local extinction of main native competitors *A. aptera* and *C. moseleyi*, *etc.*). In addition, the rate of the morphological changes observed at the Kerguelen Islands during the invasion of *C. vicina* was found to be much higher than the changes recorded over a longer period in a native continental population (**ARTICLE II**). Such rapid changes in the invaded range may result from two interacting factors.

First, evolutionary changes and rapid local adaptation may have occurred, as reported for instance in wing morphology of invasive drosophilids after their arrival in new environments (Huey et al. 2000, 2005). Insular, windswept and alpine environments are thought to stimulate the evolution of dispersal strategies by altering the balance between costs and benefits of flight, towards favoring the apparition fligthlessness in extreme cases (Medeiros & Gillespie 2011). Strong selection pressures have contributed to the evolution of flightlessness in most native sub-Antarctic insects (see Schermann-Legionnet et al. 2007; **ARTICLE II**), and may also apply to recently introduced insects that are almost all active flyers (Schermann-Legionnet et al. 2007). Changes in morphological parameters associated with flight may reflect evolutionary alterations of the costs and benefits of dispersal (Van Dyck & Matthysen 1999). Therefore, the allochronic changes of the wing of *C. vicina* may relate to altered trade-offs under the new conditions. Released selection pressures for flight remain implausible in *C. vicina*, as this species is not restricted to coastal habitats with massive food, but is also observed flying during summer, towards remote inland sites where the patchy and transient resource promotes long-range colonists (Hanski 1987). The alteration of wing morphology, together with the presence of *C. vicina* both in habitats where long-range dispersal may or may not be obligatory, suggests that *C. vicina* may back both horses at the Kerguelen Islands.

Second, *C. vicina* was repeatedly introduced at the Kerguelen Islands (Chevrier 1996). Its transportation from Europe (either from Le Havre in France or Sandefjord in Norway) has already been reported by Aubert de la Rüe in 1930, before the installation of the research station. Other individuals were probably transported from Indian Ocean and harbors of the Mozambique Channel during the next decades, then from Marseille and Île de La Réunion, because of ships anchoring at Port-aux-Français, or food and material importation. The naturalization occurred in 1978 (Chevrier 1996), and despite stringent biosecurity measures and comparatively low visitation of the Kerguelen Islands, transportation of *C. vicina* propagules probably still occurs from various locations. Considering that the first naturalized population should have suffered bottleneck and founder effects, thereby reducing its genetic diversity, subsequent drift or introductions of novel alleles may strongly impact the genetic variance within the invaded range (Sax et al. 2007). While these changes are not necessarily adaptive, they might impact the phenotypes expressed and provide additional genetic variance, which in turn may assist responses to local selection regimes (see Sax et al. 2007). However, the special isolation of the Kerguelen Islands from geographical and human frequentation standpoints considerably lowers such gene flows, in comparison to less remote invaded ranges.

Of note, we observed exceptionally high numbers of flightless individuals in two other invasive insects that are active flyers in their native range, the fly *F. maritima* and the aphid *Myzus ascalonicus* (Hemiptera, Aphididae), the latter being exclusively parthenogenetic at the Kerguelen Islands (Hullé et al. 2003b). In several sites, high proportions of adult *F. maritima* and winged individuals of *M. ascalonicus* (60-70%) were actually incapable of flying because of developmental issues (closed wings), damaged and torn out wings during adult life, or possible genetic flaws in winged aphids that even lacked several wing veins (Buchard C., Hullé M. & Laparie M., unpublished data). Yet, the flightless individuals of *F. maritima* were observed breeding on seaweeds, just as their fully winged conspecifics, with no evidence for biased sexual selection towards one phenotype or the other. The flightless winged individuals of *M. ascalonicus* can also reproduce through parthenogenesis, and the high proportion of defective wings in their populations may be studied in the light of developmental stability issues, as all phenotypes are found among clones. These two species are amongst the most widespread species at the Kerguelen Islands, and encountered a great invasive success (Hullé et al. 2003b, Frenot et al. 2005; **ARTICLE I**), so that the defective phenotypes might result from lowered selection pressures for efficient active dispersers. This view remains to be tested by comparing levels of variation within populations. Both species exclusively feed on massive, renewable and non-patchy resources, so that the same striking phenomenon cannot be reasonably expected in *C. vicina*, namely because this fly also develops in patchy and transient habitats. These observations are still preliminary, and data are currently being analyzed in both *F. maritima* and *M. ascalonicus*. These cases deserve further investigation, with special emphasis on molecular tools to decipher the possible evolution of flightlessness in sub-Antarctic Islands.

4.1.2. Physiological plasticity to salinity assisted the success of M. soledadinus

While being characterized by paucispecific communities of invertebrates, sub-Antarctic islands are biodiversity hotspots for marine vertebrates (namely seabirds and seals) that breed in large colonies in coastal areas. Alongside decaying seaweeds daily accumulated along strandlines, these numerous animal colonies produce massive and renewable organic matter, subsequently recycled by saprophagous organisms that dominate native communities of invertebrates (Smith 1977, 1978, Crafford et al. 1986, Chown et al. 1998, Vernon et al. 1998). This prominent nutrient flow from marine origin to terrestrial systems is a driving factor for the dominance of saprophagy in sub-Antarctic islands and the concentration of life along the foreshore, albeit it also occurs further inland with a lower diversity (Vernon et al. 1998).

However, these coastal habitats are characterized by hypersaline conditions. Several additive factors bring salt into the habitats where saprophagous insect are gathered, from tide and spray to organic matter itself, such as decaying seaweeds or other marine osmoconformer invertebrates deposited by tide. Osmoregulatory mechanisms exist to tolerate this osmotic constraint by preventing disruption of ionic pressure of body fluids (Edwards 1982, Somero & Yancey 1997, Oren 1999, Levin & Talley 2000, Misra & Gupta 2005, Taji et al. 2006, Majumder et al. 2010).

At the Kerguelen Islands, *M. soledadinus* funds its largest populations at low altitudes, namely in herbfields (Chevrier et al. 1997), and in a belt less than 15 meters away from the sea, where its food is the most abundant (**section 3.1.**). It mainly occurs in high densities right under decaying seaweeds deposited by tide, thereby facing hypersalinity. In 2005 and 2010, new populations were also found in lower densities further inland, up to fell-fields 300 meters above sea level. Moreover, despite active search in coastal habitats in Patagonia, its native range, this ground beetle was mostly found in forests far from the sea, where salinity is null (**ARTICLE V**). This broad ecological range is consistent with the plastic physiological adjustments found in *M. soledadinus* regarding substrate salinity, namely the accumulation of osmoprotectant compounds, a widespread response to hypersalinity among organisms (Majumder et al. 2010). We report that this physiological plasticity is a central attribute in modulating the survival of this species on substrates of extreme salinities, from null level to a level twice higher than the salinity of seawater around Antarctica (which is 34‰; Thomas & Dieckmann 2002). Preliminary experiments even indicated that *M. soledadinus* survived longer exposures to hypersalinity than the native flies *C. moseleyi* and *A. aptera* under similar controlled conditions, although *A. aptera* was also capable of rapid physiological adjustments (Bical 2011; Master 2 trainee co-supervised with D. Renault during this PhD., data not shown). Our results are consistent with those of Renault (2011), who reported that adult *M. soledadinus* can survive several days in seawater, and even passively disperse by floating across sea inlets. Although they may be rare, such long-range colonists transported from littoral margins (their departure may result from submersion by tide) probably play an important role in the invasive spread of *M. soledadinus* at the Kerguelen Islands (Renault 2011), and their survival during these events at least partly relies on the physiological plasticity evidenced here.

Ecological tolerance was reported as one of the most important features of biological invasions, namely in aquatic arthropods (see for instance gammarids in Bruijs et al. 2001, Grabowski et al. 2007). Notably, *M. soledadinus* has preferentially spread along coastlines for almost a century (Chevrier et al. 1997). Despite long term field surveys, the species was observed further inland and in altitude only recently, so that in late 1990s, it was still considered to be restricted to low altitude areas (110 meters above sea level at the maximum; Chevrier et al. 1997). This recent change in its geographical distribution marks a new step in its invasion (Lalouette et al. 2011), and its physiological plasticity regarding osmotic stress is probably playing a central role in its performance in invading new habitats further from the sea. Overall, the ecological tolerance of *M. soledadinus* along the whole gradient of salinity, and its success at littoral margins, are consistent with the idea that eurytopicity is crucial for invasive species' range extension (Lodge 1993, Moyle & Light 1996). In particular, life history parameters associated with tolerance towards severe environmental conditions, namely elevated salinity, were found to be of paramount importance for invasive species to successfully establish and proliferate in new environments (Grabowski et al. 2007).

Aside from physiological responses to external salinity, it is not known how this predatory ground beetle deals with the salt content of its food items. Ingested salt may vary depending on the diet and the available food, and impose regulatory mechanisms of salt intake such as food preferences when several food types are equally available (Trumper & Simpson 1993). These authors reported regulatory feeding behavior in *Locusta migratoria*, towards an intake target for the ratio between salt and non-mineral nutrients when a range of resources with different salt contents was offered. As we experimentally observed higher predation rates by *M. soledadinus* on maggots of Diptera than on enchytraeids, which are osmoconformers (Schone 1971, Eguileor et al. 1989), despite similar energetic outcomes (**ARTICLE VI**), such regulatory behaviors may be hypothesized in the invasive predator. This question must be further investigated in *M. soledadinus* by comparing salt content of different prey species and food preferences under controlled conditions. Physiological adjustments and specific excretory pathways should also be examined in adult *M. soledadinus* fed with diets of contrasted salt contents, as gut and excretion may also have an active role in maintaining ionic balance. For instance, the hindgut can act as a storing organ for cations in beetles (Tucker 1977), including carabids (Hanzal et al. 1992). Likewise, Pedersen & Zachariassen (2002) showed that loss of extracellular water was accompanied by a loss of extracellular sodium in the carabid genus *Cypholoba*.

4.1.3. Trophic plasticity: an advantageous feature in a changing world

Diet breath was evidenced as a strong predictor of range size in insects (Beck & Kitching 2007). *Merizodus soledadinus* is a generalist predator capable of preying on numerous organisms, from springtails to native insects (Ernsting 1993, Todd 1996, 1997, Brandjes et al. 1999, Chevrier et al. 1997, Convey et al. 2010) and even large native caterpillars (**ARTICLE I**). At the Kerguelen Islands, it was also observed preying on non-native species (**ARTICLES I** and **VI**), namely the active flyers *C. vicina* and *F. maritima*, which maintain dense populations in presence of *M. soledadinus*. For instance, we found hundreds of specimens of *F. maritima* under decaying seaweeds in Port Couvreux during the austral summer 2010-2011, despite the long-term coexistence of dense (> 250 individuals/person/10 min) populations of *M. soledadinus*. Unfortunately, it is not known when *F. maritima* colonized this site, so that the extent to which it assisted the durable persistence of *M. soledadinus* after the extinction of native prey cannot be inferred. *Fucellia maritima* might have been directly introduced at Port Couvreux, namely before 1950, as human activities were the highest at Port Couvreux and Port Jeanne d'Arc before the installation of the research station of Port-aux-Français in 1950 (permanent inhabitants, farming and whaling activities, anchoring of numerous ships; Jeannel 1940, 1964, Chevrier 1996). Alternatively, the population of *F. maritima* at Port Couvreux may be a child population resultant from the invasive spread of *F. maritima* throughout the archipelago. Likewise, migration flows from other populations of *F. maritima* towards sites invaded by *M. soledadinus* may persist, thereby assisting the maintenance of high densities of the fly despite high predation pressures. In addition, both invasive flies *C. vicina* and *F. maritima* are *r* strategists, and their ability to swarm in spite of predatory and competitive pressures may facilitate the durable success of *M. soledadinus*, even when native communities of invertebrates were strongly altered by biological invasions. Their wide distribution throughout the archipelago (Fig. 12 for *F. maritima*, Fig. 4 from **ARTICLE I** for *C. vicina*) is a critical concern to consider for predicting the future expansion of *M. soledadinus* and conserving pristine habitats.

Future research should delineate the relationships that exist between the three species, as they are all expanding and proliferating at the Kerguelen Islands, and impair native populations through biotic interactions. It must be mentioned, however, that the two invasive flies are only active during summer, and *F. maritima* is restricted to littoral margins (Fig. 12) because of its diet, as opposed to *M. soledadinus*. Together with its physiological plasticity, the ability of *M. soledadinus* to shift towards alternative diets (while still fulfilling its energy requirements) and resist food deprivation (**ARTICLE VI**) helps understanding both its persistence in long-term invaded areas and its recent expansion towards novel habitats.

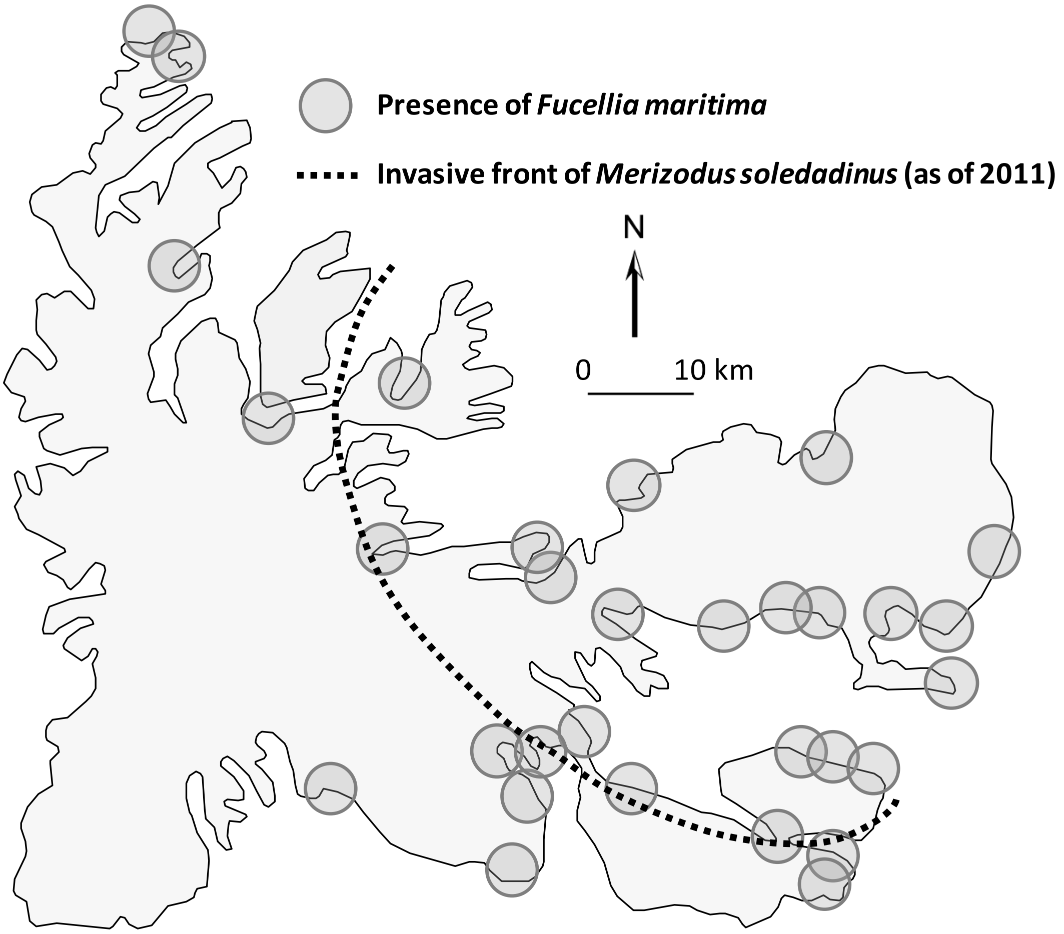


Fig. 12. Simplified map of the Kerguelen Islands (small islands not represented) with grey circles indicating locations where populations of *F. maritima* were opportunistically found during the summers 2009-2010 and 2010-2011. Most islands of the Golfe du Morbihan are also invaded by *F. maritima*, including those where human visitation is forbidden. The current general invasive front of *M. soledadinus* is represented by the black dashed curve. The figure shows that *F. maritima* has a wider geographical distribution than *M. soledadinus*, possibly facilitating its future expansion by providing massive and durable food during summer, in addition to the more vulnerable native species.

4.2. A conservation perspective: insights and reflections

4.2.1. The vulnerability of native organisms

Sax et al. (2007) suggested that competition for food or habitat seldom leads to extinctions, unlike predation. However, this assertion lacks actual demonstration and it is most likely that extinctions caused by competition are overlooked due to different time course. Native invertebrates from the Kerguelen Islands suffer both pressures as a result of biological invasions, and their joint effects lead to disruptions of communities, as already found in several sites that have long been invaded (**ARTICLE I**). The invasive *r* strategists flies *C. vicina* and *F. maritima* may outperform their native competitors during summer. For instance, *C. vicina* has a higher fecundity and growth rate than *A.aptera*, which may be advantageous in large animal colonies (Chevrier 1996, Chevrier et al. 1997). Besides coastal habitats, *C. vicina* can reach isolated patches inland (carrions of marine vertebrates that can move several hundred meters inland, or introduced mammals such as reindeer and sheep) earlier than *A. aptera* (Chevrier 1996). This author suggested that this competitive interaction may cause *A. aptera* larvae to shift towards the interface between carrion and soil, where decomposition products and liquids concentrate, but found no evidence of lowered performance (similar number of emergences with and without competition).

Nonetheless, global changes may result in – or at least stimulate – more thorough niche shifts, with negative effects on native species' performance (Klok & Chown 1997). The literature about *C. moseleyi* is scarce, but reports that the Kerguelen cabbage is its primary habitat (or even its only host; Jeannel 1964), while its occurrence beyond this plant is related to its exceptional trophic plasticity (Tréhen et al. 1987, Vernon et al. 1998). This species is capable of contrasted dietary shifts, from phytophagy on cabbages to sapro-necrophagy in animal colonies, or phycophagy in seaweeds (Tréhen et al. 1987). These alternate diets allowed *C. moseleyi* to feed on the massive organic matter accumulated along littoral margins (Tréhen et al. 1987, Vernon et al. 1998). The ability of this species to broaden its ecological range is decisive, as cabbages are threatened by climate warming, decreasing rainfalls, and invasion of rabbits (Robin et al. 2011). Vernon et al. (1998) assumed that the dietary shifts observed in *C. moseleyi* resulted from of the regression of its primary habitat. Our results suggest that this native fly may have a reduced performance at littoral margins. First, its fitness is probably lower in hypersaline decaying seaweeds, as we observed extreme mortalities in wild adults experimentally exposed to hypersaline conditions, even when originating from seaweeds (**ARTICLE VII**). We also failed at identifying specific metabotypes in individuals inhabiting contrasted environments, whereas *A. aptera* better survived hypersaline conditions and showed metabolic properties that may be advantageous in osmotically challenging environments. Second, littoral margins are currently the areas where invasive invertebrates are the most abundant. *Calycopteryx moseleyi* may thus suffer both abiotic (salinity) and biotic (mostly predation by *M. soledadinus*, but also competition with better performers) constraints in its secondary habitats. Additional experiments are currently being conducted to confirm the low physiological plasticity of *C. moseleyi* regarding osmotic conditions, and to compare it with *A. aptera* which also occurs in a variety of habitats along the gradient of salinity.

Climate changes may also directly impair life cycle and population dynamics of native invertebrates (Klok & Chown 1997). These species have evolved under the characteristic wet, cold, and buffered climate of the Kerguelen Islands, whereas the whole sub-Antarctic area is highly sensitive to climate changes, so that rainfalls, air temperature, and the proportion of freezing days are changing at a considerable rate (Bergstrom & Chown 1999; **ARTICLE I**). Native species are expected to suffer from these changes. For instance, the egg of the native fly *A. aptera* is highly vulnerable to desiccation above 11°C, and its water content can drop to 0% within only one day at such a temperature (Chauvin et al. 1987). This finding may contribute towards explaining the preferential nocturnal activity of this species (de Blignières & Tréhen 1982). Consistently, Renault & Lalouette (2011) suggested that the thermal performance of adult *A. aptera* and *C. moseleyi* matches their geographical distribution, which is restricted to high latitudes, thereby pointing to their sensitiviy to warming because of the narrow thermal preferences expected.

By contrast, from the six invasive insects currently recorded at the Kerguelen Islands (Frenot et al. 2005), the great majority is of temperate origin, or has even a cosmopolitan range (see *C. vicina, F. maritima, M. ascalonicus*, or the chironomid *Lymnophyes minimus*; Séguy 1940, Frenot et al. 2005). Individuals of *C. vicina* from the Kerguelen strain were successfully reared across a wide range of temperatures during this PhD. thesis, highlighting their broad thermal tolerance (Fig. 13; **ARTICLES I** and **II**). Consistently with the available literature (Marchenko 2001, Davies & Ratcliffe 1994, Vinogradova 1991), these experiments confirmed that individuals from the Kerguelen Islands will even benefit from rising air temperature, as it will accelerate their life cycle and facilitate the proliferation of the species during austral summer (Fig. 13). It is considered that climate change recorded in the 1970s at the Kerguelen Islands enabled the full development of *C. vicina*, by matching its ecological requirements (second scenario by Facon et al. 2006), and was a significant step of its invasion (see **ARTICLE I**). Remarkably, *M. soledadinus* is the only invasive insect exclusively originating from high latitudes, and its resistance to desiccation is relatively low (Todd & Block 1997). Nevertheless, the study of its thermal biology by Lalouette et al. (2011) demonstrated that despite its limited thermal plasticity, this ground beetle maintains activity at temperatures well above average summer conditions at the Kerguelen Islands, and thus has abundant scope to deal with future climate changes. Lalouette (2009) found a thermal optimum around 17 °C, and Lalouette et al. (2011) even indicated that warming at sub-Antarctic latitudes is releasing this species from abiotic constraints, and contributed towards the explosion of its invasion in the 1990s. The fate of native invertebrates from the Kerguelen Islands is therefore being challenged by both direct and indirect impacts of climate changes. This pessimistic statement generally stands for most oceanic islands, and also encompasses the progressive removal of abiotic barriers to future introductions into insular ecosystems (Tassin 2010).

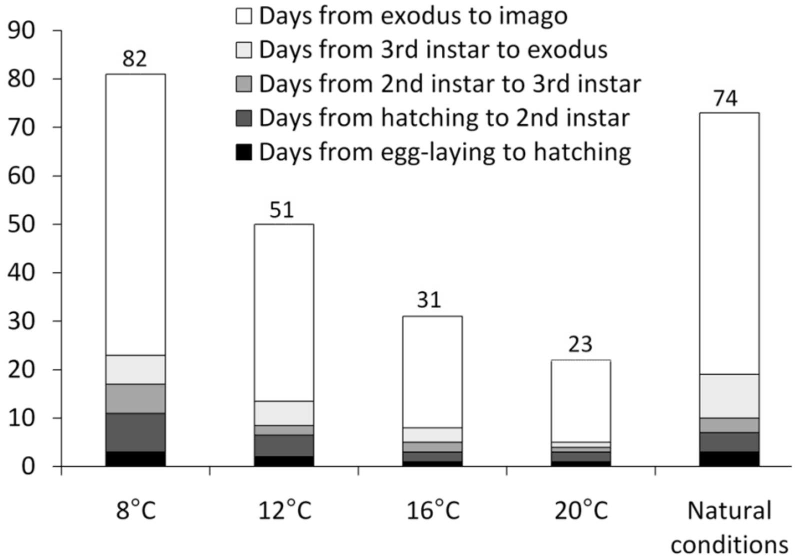


Fig. 13. Duration of development of *C. vicina* (Kerguelen strain) reared at different temperatures under controlled condition, and comparison with individuals reared outdoor at Port-aux-Français under similar experimental conditions during summer 2008-2009. Food was supplied *ad libitum* (sheep liver) for initial sample size of 30 eggs (before mortality), ensuring that overcrowding and mass effects did not bias the development (see Charabidze et al. 2011). The durations of development recorded correspond to the minimal delay necessary to observe at least one individual of the next stage.

4.2.2. The future success of invasive species at the Kerguelen Islands, and beyond

Together with the beneficial effects of climate warming previously mentioned, *M. soledadinus* and *C. vicina* both possess life traits that may further improve their invasive success and spread within the Kerguelen Islands. First, we demonstrated that *M. soledadinus* is capable of resisting extreme salinities, and can starve for periods long enough to resist passive transport (seawater, hitchhiking on expeditioneers, *etc.*). This species can also resist temperatures warm enough (Lalouette et al. 2011) to survive transportation aboard ships. Particular attention must then be considered during expeditions aboard *La Curieuse* and l'*Aventure II*, as these two ships are extensively used for logistics between Port-aux-Français and numerous remote sites of the Kerguelen Islands, some of them being almost free from alien plants or animals. Some protected islands, such as the Nuageuses in the North West of the archipelago, are even pristine to our knowledge (Tréhen et al. 1987), but they were extremely rarely visited since the discovery of the Kerguelen Islands, and they were not studied after the mid 1990s.

Long range dispersal of *Calliphora vicina* may rather rely on active flight, so that a flying adult was caught on the western Loranchet Peninsula during summer 2010-2011, although this area was thought to be far away from *C. vicina*'s range, and even beyond its ecological preferences (**ARTICLE I**). Invasions by terrestrial mammals such as rabbits, and especially reindeer, may be facilitating factors for the future spread of this saprophagous fly in western areas that are almost pristine from alien invertebrates. Populations of invasive reindeer can act as pathways for *C. vicina*, as (i) they live in nomadic groups, (ii) they occur towards western and inland areas that are not invaded by alien insects yet, (iii) their carrions represent massive food patches that cannot be fully eaten by scavenger birds, nor be quickly colonized by native wingless decomposers (Chevrier 1996), and (iv) although no eradication programme is planned yet, it would be economically difficult to apply in a large archipelago such as the Kerguelen Islands.

Moreover, the life histories of *M. soledadinus* and *C. vicina* are compatible with passive long-range dispersal towards other sub-Antarctic terrestrial habitats, namely by being transported in luggage, shoes, food (mostly for *C. vicina*), or even soil attached to containers and vehicles aboard ships (Hughes et al. 2010). The ships that visit the Kerguelen Islands may subsequently anchor in other sub-Antarctic harbors during their journey, and release the transported propagules, even if biosecurity measures are advised to mitigate the risks. The starvation resistance of adult *M. soledadinus* and the duration of the non-feeding pupal stage in *C. vicina* (see Fig. 13), all along with their resistance towards high temperatures, are key features that make secondary (and long range) transportation possible. As these species are invading the Kerguelen Islands, proliferating, and are now amongst the dominant species therein, their accidental transportation from this archipelago is becoming increasingly likely.

4.3. Methodological issues and future directions

The present work has emphasized the relevance of examining how invasive species suit their new environments in time and space to succeed, by combining morphometrics and ecophysiological approaches. However, it also raised methodological challenges pertaining to the study of natural processes and phenomena in wild animals. These challenges encompass both conceptual and methodological issues.

First, as highlighted in **sections 2.4. and 4.1.1.**, morphological variation is the integrative result of several co-existing factors, and may in turn interact with them. Drawing and resolving the relative importance of these factors on the morphological patterns observed in nature is thus difficult in sub-Antarctic islands, as field work cannot be undertaken continuously in these areas due to logistical constraints. Both *M. soledadinus* and *C. vicina* offer exceptional opportunities to tackle real-time evolutionary processes, but experiments of reciprocal translocation would be necessary to address the relative roles of evolutionary mechanisms and phenotypic plasticity, by testing whether the observed changes are reversible or permanent, and heritable or not. Such experiments would imply ethical issues (Sax et al. 2007). Molecular tools must also be combined to the present results to establish the genetic basis for the patterns observed. Second, rearing *M. soledadinus* at the laboratory would open a window to address numerous problematics raised by the present work. At the moment, all attempts to rear this species under controlled condition failed, but additional efforts are required to achieve this task. Rearing *M. soledadinus* and improving the knowledge of its life cycle in nature are tightly and reciprocally inter-related, as progress in one topic will result in progress in the other, and ultimately found a fertile basis for facilitating fitness-related questions in this species. Breeding this species in the laboratory would allow us to control parameters that cannot be monitored in the field, and to accurately elucidate the relationship that exist between morphology, salinity of habitat or diet, temperature, availability and quality of resources, and fitness outcomes. As a first step towards fulfilling this priority, a survey is currently being conducted at the Kerguelen Islands to measure on a semimonthly basis the evolution of reproductive activity, fecundity of females, and sex ratio throughout the year. The data shall be collated to data available for *M. soledadinus* from South Georgia, where its invasive success and distribution extent are comparatively low.

In addition, we believe that the combination of field-based and experimental studies that were used in the present work must receive special attention and be further extended, as it is the most consistent way of directly measuring the actual consequences of a parameter in nature. Measuring life history traits in individuals experimentally acclimated to a specific factor prior to the release in semi-natural conditions would considerably improve our understanding of its outcomes on the performance of *M. soledadinus* at the Kerguelen Islands. Predicting the range expansion of *M. soledadinus* is a central concern, but remains a complex task as the performance of this insect directly depends on biotic and abiotic constraints that can change dynamically. The trophic status (a continuum from satiated to starved), reproductive status, senescence, temperature, or salinity must be addressed with special emphasis on their outcomes on functional traits such as fecundity, lifespan, and dispersal power and activity. Predicting the future expansion and impacts of *M. soledadinus*, or even using this case study to build models *in silico*, implies for instance to test whether its dispersal activity correlates with the availability and quality of resources, and whether colonists by residents ratio is altered over residence time. Videotracking methods are currently being used to answer the first part of this question, but complementary field experiments will be required to validate the results, as examining dispersal power and activity at the laboratory can only bring preliminary insights of what may actually happen in real habitats.

From a general perspective, the study of the significance of the yet limited but measurable human activities in spreading aliens deserves special attention at the Kerguelen Islands. During this PhD. and the austral summer 2009–2010, a broad survey has been initiated aiming at accurately measuring in several locations of the archipelago the impact of human pedestrian, hiking, or logistical (huts, supply) activities on the number of locally naturalized species. Data are already collected for both plants and invertebrates in numerous locations that range from extreme human visitation to almost no human presence in decades. The Kerguelen Islands are an unrivalled model for addressing this problematics at a microscale, as (i) biota is depauperate and comparatively easy to understand, and most importantly, (ii) the low human frequentation in every site is historically detailed and archived right up to the present day, with accurate number of visitors, date and duration of visitation, purpose, *etc.* These unique data were completed during the austral summer 2010-2011 to extend the geographical range investigated within the archipelago. Alongside the international programme *Aliens in Antarctica* presented in **section 1.2.2.2.** of this dissertation, the goal of this study will be to improve our knowledge of the extent and implications of introductions in the Southern Ocean Islands (reviewed in Frenot et al. 2005).

Altogether, our results have provided significant information for better understanding the long-term success of invasive species within their new habitats. However, the present work raised even more questions that remain to be addressed. There are still many lessons to learn from the success of *M. soledadinus* and *C. vicina* at the Kerguelen Islands (*F. maritima* should also be considered in future research). Furthermore, the arguments advanced here about the reasons for the success of invaders rest upon study of winners, not losers, as in most studies of invasions. However, focusing on losers as well would be the best way to identify particular features that are essential to invasion success and make the difference between winners and losers. The future directions of research proposed may yield answers to the challenging question of how the underlying mechanisms of invasive success interplay. Because of the particular situation of the Kerguelen Islands, and the comparatively low levels of human activity outside the research station, these invasions that were monitored since introduction are fruitful models to thoroughly unravel how invaders relate with their new environment over the long term, and spread with no (or reduced) further human assistance.

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ANNEXE 1 – RÉSUMÉS EN FRANÇAIS DES TRAVAUX PRÉSENTÉS

Article I

The significance of the sub-Antarctic Kerguelen Islands for the assessment of the vulnerability of native communities to climate change, alien insect invasions and plant viruses

L'ensemble des environnements et des modifications d'origine anthropique dans les îles subantarctiques offre des opportunités exceptionnelles pour améliorer notre compréhension des conséquences potentielles des changements climatiques et des invasions biologiques sur les écosystèmes terrestres. L'impact des espèces invasives sur les organismes autochtones, ainsi que la facilitation de l'étape de naturalisation grâce au réchauffement climatique, sont bien documentés dans l'archipel français des Îles Kerguelen (Sud de l'Océan Indien). Cette étude présente un aperçu de la vulnérabilité des communautés subantarctiques terrestres face à l'interaction entre les changements climatiques et les espèces introduites. Nous couplons des données issues d'un programme mené depuis 1974 aux Îles Kerguelen à une synthèse de la littérature pour étudier le mécanisme et l'impact des invasions biologiques dans cette région. D'abord, nous nous attachons aux tendances climatiques récentes dans la région Antarctique, ainsi qu'à leur influence potentielle sur la naturalisation, la distribution et l'abondance des insectes introduits. Nous abordons la distribution d'espèces introduites ayant pourtant une variance génétique limitée aux Îles Kerguelen. Enfin, nous caractérisons la vulnérabilité des communautés natives aux espèces introduites en utilisant comme exemples d'espèces introduites une mouche, un carabique et cinq espèces de pucerons, les pucerons pouvant également avoir un impact indirect en constituant des vecteurs de virus de plantes. Les enseignements tirés de l'exemple des îles subantarctiques peuvent être appliqués aux systèmes continentaux plus complexes des régions tempérées et aider au développement de mesures internationales pour minimiser l'impact des espèces introduites.

Article II

When aliens meet novel selection pressures: morphological change of the wing in a flying insect invading a world of flightlessness

La mouche cosmopolite *Calliphora vicina* a été naturalisée dans l'archipel subantarctique des Îles Kerguelen à la fin des années 1970, suite à une période de réchauffement climatique lui permettant de compléter son cycle de développement. Bien que la température et le vent puissent limiter son activité de vol, cette espèce a envahi l'archipel jusqu'à des sites distants du point d'introduction. La plupart des compétiteurs natifs pour la nourriture ont convergé vers l'aptérisme ou l'inaptitude au vol sous les conditions subantarctiques, la capacité de vol de *C. vicina* pourrait donc être à la fois un handicap ou un avantage au regard des changements climatiques actuels. Nous avons utilisé la morphométrie géométrique pour déterminer si l'aile de cette espèce a subi des modifications au cours du temps au sein de l'archipel (1998 *vs.* 2009), ceci en comparaison d'une population continentale de l'aire native (1983 *vs.* 2009). Les variations consécutives au développement à différentes températures en conditions contrôlées ont également été étudiées dans les deux populations. Nous montrons chez les individus d'élevage des normes de réaction à la température différentes entre les populations à la fois pour la taille et la forme de l'aile, avec une amplitude de variation de forme plus importante dans la population de Kerguelen. Le changement allochronique de forme le plus important a été observé chez les femelles sauvages de Kerguelen, malgré un délai entre les périodes d'échantillonnage plus court que dans la population continentale. Nos résultats montrent les changements rapides de l'aile de *C. vicina* aux marges de son aire de distribution, au cours de son invasion. Ces changements rapides, ainsi que la plasticité phénotypique de l'espèce, sont des éléments intéressants du point de vue évolutif compte tenu de la règle de l'aptérisme (ou de l'inaptitude au vol) observée chez les insectes natifs de la région subantarctique.

Article III

Variation of morphometric traits in populations of an invasive carabid predator (*Merizodus soledadinus*) within a sub-Antarctic island

Les prédateurs invasifs peuvent changer leurs propres ressources trophiques en déplaçant les proies natives ou en réduisant leur diversité et leur abondance. La qualité et la disponibilité de la nourriture étant deux facteurs essentiels de la taille adulte chez les arthropodes, l'altération des ressources trophiques disponibles par les prédateurs invasifs pourrait affecter leur propre morphologie. Le carabique prédateur *Merizodus soledadinus* a été accidentellement introduit en un site unique des Îles Kerguelen en 1913. Sa propagation a été suivie sur le long terme, offrant une opportunité de recherche exceptionnelle avec la formation de plusieurs populations dans des habitats similaires, différant seulement par le temps de résidence de *M. soledadinus*. Pour tester la relation entre la morphologie et le temps de résidence de *M. soledadinus* dans chaque site, nous avons mesuré neuf traits morphologiques dans cinq populations distinctes. Des différences morphologiques significatives ont été détectées : les individus issus des premiers sites colonisés sont en moyenne les plus petits, tandis que ceux des sites colonisés récemment sont en moyenne les plus gros. L'étude met également en évidence des variations entre sites du dimorphisme sexuel pour le dernier sternite abdominal : sa longueur varie en fonction du site pour les femelles et non pour les mâles. La diminution de taille de *M. soledadinus* est discutée à la lumière d'effets *a priori* (développement en conditions de restriction trophique, survie) et *a posteriori* (compétition intrapopulation, cannibalisme) sur la croissance et le développement.

Article IV

Is dispersal promoted during range expansion of invasive species? A synchronic approach using morphometrics in a ground beetle invading sub-Antarctic islands

Lorsqu'une espèce invasive est naturalisée dans un nouvel environnement, sa propagation peut promouvoir la capacité de dispersion des individus par des effets directs ou indirects de la sélection, accélérant ainsi l'expansion de l'espèce et augmentant son impact. La morphologie est liée à la dispersion dans de nombreux taxons et représente un paramètre intégrateur des changements temporels et géographiques de la capacité de dispersion. En utilisant les données morphologiques synchroniques de Laparie et al. (2010), nous avons étudié la relation quantitative entre le temps de résidence et une combinaison multivariée des paramètres morphologiques mesurés chez *Merizodus soledadinus*, un carabique accidentellement introduit en un site unique des îles Kerguelen en 1913. Nous décrivons dans cette étude une différentiation morphologique cohérente avec l'idée d'une promotion de la capacité de dispersion selon le temps de résidence, depuis la population fondatrice aux populations plus jeunes. Les différences morphologiques entre les populations s'accordent quantitativement avec leurs différences de temps de résidence, représentant ainsi un gradient d'augmentation de taille des traits liés à la dispersion au cours de la colonisation de l'archipel par l'espèce. Ces résultats constituent une base intéressante pour l'étude de la sélection spatiale et de la sélection des disperseurs le long du front d'invasion des espèces, puisqu'ils mettent en évidence la nature dynamique de la capacité de dispersion au cours des invasions biologiques.

Article V

Metabolic fingerprinting and molecular responses to salinity in the invasive ground beetle *Merizodus soledadinus* at the Kerguelen Islands: do physiological plasticity and ecological range match?

La salinité est un paramètre abiotique qui influence la survie et la fitness des insectes terrestres. Ce paramètre peut induire des perturbations osmotiques et ioniques, que l'organisme peut compenser en accumulant des solutés compatibles. Le carabique invasif *Merizodus soledadinus* (Coleoptera, Carabidae) se disperse principalement le long du littoral aux Îles Kerguelen. Il doit donc supporter les variations de salinité associées à ces habitats côtiers. Dans son aire naturelle, en Patagonie, ce carabique est le plus souvent retrouvé en forêt et le long des rivières, soit des habitats dans lesquels la salinité est considérablement plus faible que dans les habitats littoraux aux Îles Kerguelen. Nous avons étudié les ajustements métaboliques des adultes de *M. soledadinus* exposés à différents niveaux de salinité (0, 35 et 70‰) à 4 °C (température moyenne annuelle aux Îles Kerguelen) ou 8 °C (température moyenne estivale aux Îles Kerguelen). Nous avons fait l'hypothèse que les adultes de *M. soledadinus* sont capables d'une plasticité physiologique suffisante pour supporter une grande amplitude de variations de salinité grâce à l'accumulation de solutés compatibles. L'analyse des profils métaboliques (GC-MS) a montré (i) la diminution des concentrations en métabolites intermédiaires (TCA) lorsque la durée de l'exposition augmente et (ii) l'accumulation de solutés compatibles (erythritol, sorbitol et inositol), d'acides aminés (isoleucine, proline et glutamine) et d'acide pipécolique lorsque les individus sont exposés aux conditions de salinité les plus intenses (70‰). Ces solutés compatibles ont probablement un rôle dans la restauration de l'osmolalité de l'hémolymphe et le maintien des processus d'osmorégulation. Nous avons également utilisé une approche moléculaire complémentaire pour mesurer la variation de l'expression de HSC70 chez *M. soledadinus* à des salinités de 0, 35 et 70‰ (4 et 8 °C). L'expression de HSC70 diminue significativement lorsque la salinité augmente, mais n'est pas température-dépendante.

Article VI

Starvation resistance and effects of diet on energy reserves in a predatory ground beetle (*Merizodus soledadinus*; Carabidae) invading the Kerguelen Islands

La relation entre les besoins nutritionnels et la disponibilité et la qualité de la nourriture est un paramètre déterminant de l'expansion géographique des espèces invasives. Dans l'archipel subantarctique des Îles Kerguelen, le carabique invasif *Merizodus soledadinus* devient l'invertébré prédateur principal dans les habitats qu'il colonise, conduisant à l'extinction locale de plusieurs espèces de mouches natives. De tels changements de la structure des communautés de proies peuvent altérer la gestion de l'énergie (stockage et dépenses) de ce prédateur. Chez cette espèce, nous avons suivi la survie et la masse corporelle lors de la restriction trophique et évalué les effets de deux régimes distincts (asticots de mouches ou enchytréides) sur la consommation et la restauration des réserves corporelles (sucres et triglycérides). Nous avons mis en évidence une capacité de jeûne pouvant aller jusqu'à plus de 60 jours chez les adultes, ceux-ci se nourrissant tous les 3.76 jours en moyenne lorsque la nourriture est disponible. Un taux de prédation plus fort sur les asticots que sur les enchytréides a été observé, associé à des variations de masse corporelle d'une plus grande amplitude. Les sucres et les triglycérides ont été significativement consommés lors de la privation de nourriture et restaurés après restitution de la nourriture, mais ont varié de manière similaire entre les individus nourris avec asticots ou enchytréides. D'autres paramètres pourraient déterminer les préférences alimentaires observées, telles que la teneur en sel dans les tissus des proies, puisque *M. soledadinus* se nourrit essentiellement dans les habitats hypersalins du littoral et pourrait donc limiter la consommation d'organismes osmoconformes.

Article VII

Habitat phenotyping of two sub-Antarctic flies by metabolic fingerprinting: evidence for a species outside its home?

La métabolomique peut contribuer à identifier les réarrangements de réseaux métaboliques chez les organismes exposés à des conditions environnementales variables. Le maintien de la performance d'un organisme peut impliquer des changements de flux et de voies métaboliques, conduisant à des signatures métaboliques spécifiques aux habitats. Plusieurs insectes des îles subantarctiques françaises, incluant les mouches aptères *Anatalanta aptera* et *Calycopteryx moseleyi*, sont exposés à des conditions hypersalines lorsqu'ils se nourissent de la matière organique d'origine marine accumulée le long du littoral (laisses de mer notamment). Cependant, l'espèce *C. moseleyi* a longtemps été considérée comme inféodée au chou de Kerguelen, qui est distribué depuis le littoral jusqu'à des altitudes élevées dans les reliefs à l'intérieur des terres. Par conséquent, les fortes densités de populations de *C. moseleyi* observées dans les zones hypersalines où les algues en décomposition s'accumulent sont surprenantes. Elles pourraient impliquer des mécanismes d'osmorégulation tels que l'accumulation de solutés compatibles. Nous avons étudié les métabotypes quantitatifs (phénotypes métaboliques) entre des individus de *C. moseleyi* sauvages prélevés sur laisses de mer (hypersalinité) et sur choux de Kerguelen de zones non-salines (éloignés de la zone intertidale). Les métabotypes d'individus sauvages d'*A. aptera*, la principale espèce compétitrice de *C. moseleyi* pour la ressource trophique sur laisses de mer, ont également été examinés. Le traitement statistique, visant pourtant à maximiser les différences entre les groupes, a révélé des similarités importantes entre les métabotypes des individus de *C. moseleyi* issus de laisses de mer et ceux des individus issus de choux de Kerguelen non salins, malgré des morphotypes, des conditions de salinités et des régimes trophiques contrastés. *Anatalanta aptera* se caractérise par des concentrations plus importantes en glycérol, inositol et autres solutés compatibles pouvant contribuer à sa meilleure performance dans les environnements hypersalins. Les laisses de mer et algues en décomposition pourraient représenter une niche secondaire (sub-optimale) chez *C. moseleyi*, favorisée par la régression importante du chou de Kerguelen suite aux changements climatiques et à la pression d'herbivorie causée par l'invasion du lapin aux Îles Kerguelen.

RÉSUMÉ

Le succès des espèces invasives dépend de l'adéquation entre leurs traits d'histoire de vie et les caractéristiques environnementales (biotiques et abiotiques) de leurs nouveaux habitats. Le succès invasif d'une espèce peut donc reposer sur sa pré-adaptation, être déclenché par la levée de certaines pressions sélectives, les perturbations, ou encore la réponse rapide de l'organisme aux nouvelles contraintes sélectives. La plasticité phénotypique et les processus évolutifs sont ainsi des paramètres essentiels dans les invasions biologiques, en faisant des situations privilégiées pour observer les processus écologiques et évolutifs en temps réel. Nous nous sommes intéressés aux réponses morphologiques et écophysiologiques exprimées dans le temps et l'espace au cours de l'invasion des Îles Kerguelen (archipel subantarctique) par le carabique prédateur *Merizodus soledadinus* et la mouche saprophage *Calliphora vicina*, dont les stratégies de vie sont contrastées. Nous montrons la différenciation morphométrique de populations de *M. soledadinus* en fonction de leur temps de résidence, ainsi que les changements rapides de la morphologie de l'aile de *C. vicina* dans ces îles où l'inaptitude au vol est la règle. Une attention particulière est accordée au rôle de la plasticité phénotypique de *M. soledadinus* dans la colonisation de nouveaux habitats (plasticité physiologique à la salinité) et dans son maintien durable malgré l'impact qu'il exerce sur la disponibilité des proies (plasticité trophique). Ces ajustements à plus ou moins long terme sont des éléments déterminants du succès invasif de ces deux insectes à mesure qu'ils progressent et rencontrent de nouveaux régimes de sélection.

Mots-clefs : *Calliphora vicina*, écophysiologie, évolution, île subantarctique, invasion biologique, *Merizodus soledadinus*, morphométrie, plasticité phénotypique

ABSTRACT

The success of invasive species depends on the adequacy between their life history traits and the environmental characteristics (biotic and abiotic) of their new habitats. The invasive success may then rely on pre-adaptation, be triggered by the release of some selection pressures, perturbations, or quick responses of the organism to the new selection pressures. Phenotypic plasticity and evolutionary processes are then prime components in biological invasions, so that invasive species can be considered as key models for monitoring ecological and evolutionary processes in real time. We thus investigated morphological and ecophysiological responses produced in time and space during the invasion of the sub-Antarctic Kerguelen Islands by the predatory ground beetle *Merizodus soledadinus* and the saprophagous blowfly *Calliphora vicina*, which possess contrasted life strategies. We show morphological differentiation among populations of *M. soledadinus* depending on their residence time, as well as rapid changes of the *C. vicina*'s wing morphology in these islands where flightlessness is the rule. The invasion of *M. soledadinus* was studied with special emphasis on the role played by phenotypic plasticity in colonizing habitats that differ from native ones (physiological plasticity to salinity) and maintaining durable populations despite the negative feedback of this predator on the availability of its own prey (trophic plasticity). As they spread and encounter novel selection regimes, these adjustments at different timescales are of paramount importance in the invasive success of both these insect species.

Keywords: *Calliphora vicina*, ecophysiology, evolution, sub-Antarctic island, biological invasion, *Merizodus soledadinus*, morphometrics, phenotypic plasticity