Phenotypic and genetic characterisation of the carabid beetle Merizodus soledadinus along its invasion gradient at the subantartic Kerguelen Islands

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« L'Homme est fils de la forêt et père du désert »

René Jeannel

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General Introduction

1 - Biological invasions and associated concepts

The distributional ranges of many species fluctuates naturally on a time scale of centuries to years, linked with alterations in climate or biological interactions. Human-mediated transportation and introduction of propagules outside of their natural biogeographic range have increased the opportunities for plants and animals to cross geographic boundaries by orders of magnitude (Brown & Sax, 2004; Cassey et al., 2005). In particular, since the industrial revolution and the demographic explosion of human population, global trade accelerated this phenomena, by multiplying the vectors of transportation among distant regions. This has resulted in increasing number of examples of newly arrived species becoming dominant in the introduced community (Mack et al., 2000; Bax et al., 2003; Roy et al., 2011), expanding geographic ranges (Simmons & Thomas, 2004; Phillips et al., 2010; White et al., 2012), and impacting autochthonous communities (Hejda et al., 2009; Vilà et al., 2011). Biological invasions are now recognized as a major threat to biodiversity alongside with global climate change, habitat fragmentation and modification of land use, and greatly participate to biotic homogenization (Lodge, 1993; Vitousek et al., 1996; Dukes & Mooney, 1999; McKinney & Lockwood, 1999; Millenium Ecosystem Assessment, 2005; Simberloff et al., 2013).

Biological invasions received a growing interest from biologists over the past decades (at the instigation of the SCOPE program in 1982), and constitute now a research field *per se*. Different aspects of invasions are currently investigated, with different objectives. Studies for conservation purpose have been intensively developed, to understand how invasive species can be managed, but they also are associated to other research fields because they constitute natural experiments ideal to study ecological and evolutionary processes in real time, like fast-growing populations, shifts among ecological niches, and accelerating expanding ranges (Sax et al., 2007; Davis, 2009).

Literature related to biological invasions extensively reported invasion cases. This overwhelming number of studies greatly varies in scopes and methods, depending on the main research question asked (impacts on native population and ecosystem, comparison among introduced and source populations, population dynamics, reasons underlying invasive success, etc…) and on the taxa studied (plant, or animal, terrestrial or aquatic, sessile or motile, predator or phytophagous). This profusion of studies led to parallel and confusing development of concepts and terminology related to biological invasions (reviewed in Falk-Petersen et al., 2006; Valéry et al., 2008; Catford et al., 2009; Van Kleunen et al., 2010; Blackburn et al., 2011; Gurevitch et al., 2011). As a result, most of studies related to biological invasions specify which definition of invasive species they use. This confusion may also partly result from the human perception of non-native, introduced, alien, established, invasive species (Larson, 2005; Tassin & Kull, 2015), often considered as being 'bad'. Yet, during the last decade, several unified frameworks have been proposed to better describe biological invasion processes and further

elaborate generalized theories and concepts (Shea & Chesson, 2002; Facon et al., 2006; Catford et al., 2009; van Kleunen et al., 2010; Blackburn et al., 2011).

a) *Terminology and concepts of the invasion process*

Williamson (1996) and Richardson (2000), respectively focusing on animals and plants, generalized the invasion process as a sequence of a variety stages or environmental barriers that propagules successfully cross, before being successfully established and potentially invasive in their introduced environments (Williamson, 1996; Williamson & Fitter, 1996; Richardson et al., 2000). Three main stages are now widely recognized by the scientific community when describing invasion patterns:

- The transport stage: propagules sampled in the native area have to survive during the transfer to the new area where they will be released (or where they will escape),
- The establishment stage: propagules introduced in a new area must survive and reproduce to establish a viable, self-sustaining population. This stage is often associated with lag periods, because establishment may require multiple introductions or adaptation to the new environmental biotic and abiotic conditions,
- The invasion stage, where propagules successfully increase in demography and expand range boundaries in the introduced environment. This phase is often rapid and exponential.

The Fig.1 from Blackburn et al. (2011) synthetises principal stages and barriers encountered by propagules/alien species during an invasion process.

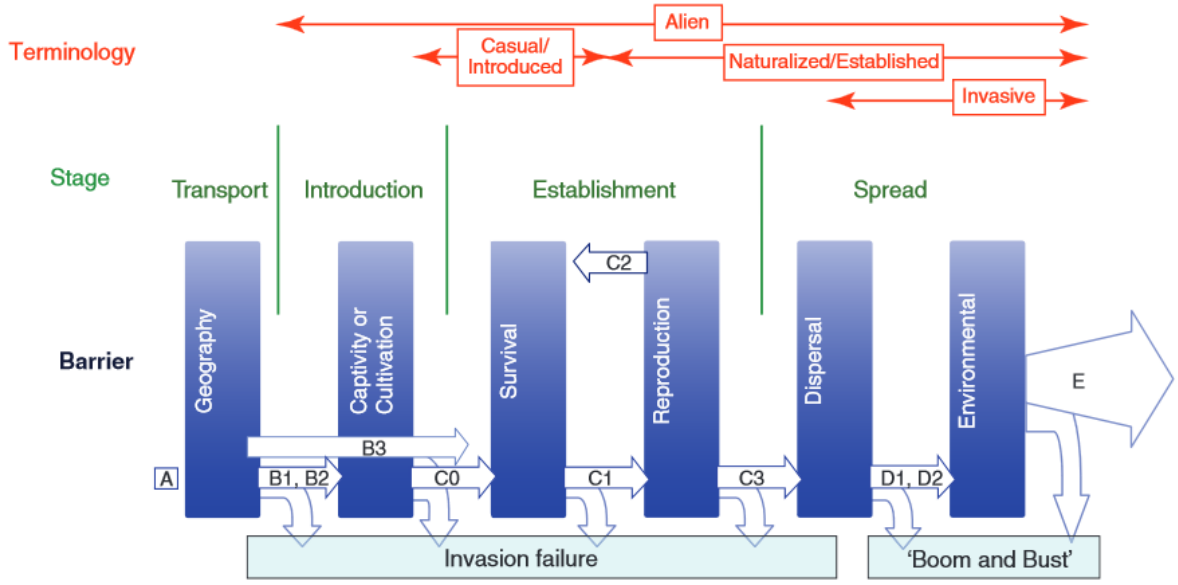

Fig. 1 General frame of the successive stages and barriers propagules can face during an invasion process, starting from the sampling of the specimens in their native area up to their introduction into a new geographic region. Terminology associated to each invasion stage is mentioned. If one of this stage is not passed successfully the invasion process may fail (modified from Blackburn et al., 2011)

Given the current intensity of global trade, it is likely that the number of introductions (estimated from the number of unique occurrence of species outside their geographical range, Carlton & Geller, 1993) is very high. Comparatively, few exotic specimens establish populations, and even

fewer become invasive. Illustrating the low probability for a population to become invasive, Williamson's Ten's rule (1996) estimates that 10% of the propagules released in a new area successfully establish a viable population, and among them 10% can become invasive. This generalization has been largely debated and should rather be considered as an example (see Jeschke & Strayer, 2008) of the strength of the filters determining the success of an invasive species.

b) *Going further than invasiveness and invasibility*

A major issue in the field of biological invasion is the ability to predict which introduced species will become invasive (Mack et al., 2000; Leung et al., 2004; Williamson, 2006; Romanuk et al., 2009). Authors tried to resolve this question by analysing if there are biological traits shared by invasive species, and, in parallel, if there are similarities among environmental characteristics of the invaded habitats (Lodge, 1993; Lonsdale, 1999; Kolar & Lodge, 2001).

The characteristics of a "good invader", or invasiveness, include life history traits that favour fast reproduction in order to rapidly establish viable populations, high reproductive rate, autogamy or vegetative reproduction, diet breadth, small body size, and high competitive abilities against native communities that allow establishment and subsequent potential spread (Kolar & Lodge, 2001).

Native communities in the introduced area might be particularly vulnerable to invasion, termed 'invasibility' of the recipient community, especially if ecological niches are poorly diversified (although criticized by Levine & D'Antonio (1999). Indeed, an 'unsaturated' environment increases the likelihood for a new species to readily establish a viable population, if other biotic and abiotic factors sustain its persistence (Hierro et al., 2005). In this line, insular ecosystems are considered as being particularly sensitive to the effects of invasive species because, as depicted by the high number of exotic species generally recorded on islands. The high level of endemicity of island communities, which, in several instances, evolved under lower biotic pressures as compared with continental communities (see for instance the famous example of the brown tree snake *Boiga irregularis* accidentally introduced in Guam decimating the avian forest community, naïve to tree climbing predators, Wiles et al., 2003). Invasibility of the recipient habitat may vary depending on the invasive species studied (Reaser et al., 2007), whose specific ecological requirements vary. Meanwhile, habitat disturbance is globally recognized as an important feature of susceptibility to invasive species (Lozon & MacIsaac, 1997), and especially on islands (D'Antonio & Dudley, 1995). Anthropogenic disturbance holds a special place in a fundamental debate on invasive species: the correlation between dominance of invasive species and decline of native populations does not constitute an evidence that ecological changes are driven by invasive species (Didham et al., 2005). Indeed, habitat disturbance or fragmentation could have both detrimental impact on native populations abundance and diversity while being beneficial for exotic species, then labelled as "passengers" of ecological change. MacDougall & Turkington (2005) empirically tested this driver or passenger characteristics of invasive grasses in fire-suppressed oak savannah of British Colombia. They hypothesized that reducing or

removing invasive grasses would induce native community resilience in the case of a 'driver' model, as invasive species would be dominant over the native community due to direct competition, or would have very little effect in the case of a 'passenger' model, as invasive species benefit from unavailable resources or constraining disturbance conditions for native species. The results of this study were among the first to point toward the passenger model and their framework allowing to empirically test the effects of habitat disturbance and invasive species on native population declines have then been applied on other invasion cases (Jäger et al., 2007; Hermoso et al., 2011; Grarock et al., 2013). Results are inconsistent, as expected from different invasive species/invaded ecosystems scenarios. Didham et al. (2005) suggested that interactions between drivers of ecological changes (habitat disturbance and fragmentation, invasive species, historical drivers) would probably be a more realistic view of this question.

To conclude, searching for lists of traits related to invasibility and invasiveness was often inconclusive (Mack et al., 2000) and only applicable to few taxonomic groups (Kolar & Lodge, 2001). This extensive work revealed the large variety of invasive strategies, depending on the habitat characteristics and species' traits. Invasion success is thus currently viewed as a match between a species and a system at one point rather than by a suite of specific traits exhibited by a species in regard to main characteristics of introduced communities (Lodge, 1993; Catford et al., 2009). For example, climate matching between the introduced area and the native range of an alien species will facilitate invasion success because of the pre-adaptation of the species to not-so-new abiotic conditions.

c) *Ecological and evolutionary factors driving the invasion success / failure*

Rooted in the ideas of the two Charles (Darwin, 1859 and Elton, 1958), several hypotheses have been raised to explain invasion success or success (Davis et al., 2000; Richardson & Pyšek, 2008; Catford et al., 2009; van Kleunen et al., 2010). Fig. 2 integrates the main hypothesises currently available in the literature.

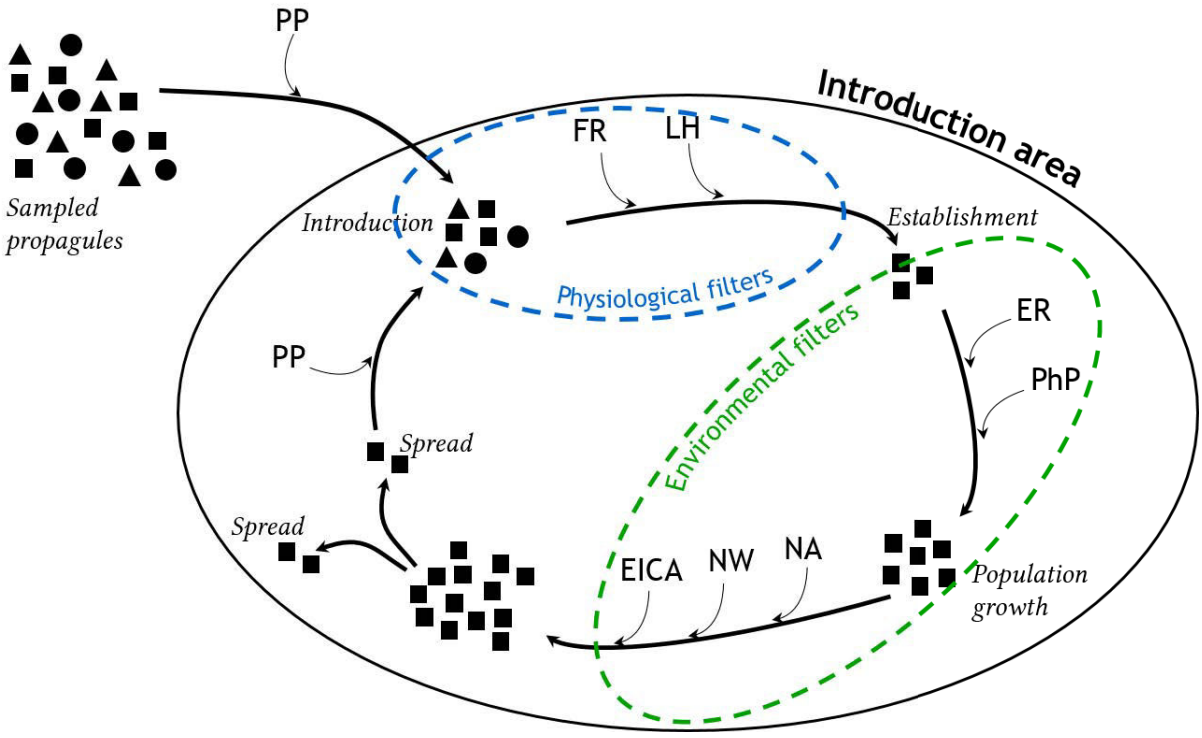

Fig. 2 Conceptual framework of major hypotheses driving invasion success [Adapted from van Kleunen et al., 2010]. This framework represents an ecological continuum, from the sampling of the propagules from their native area until their geographical spread into the introduced geographic region. The blue shaded oval ('Physiological filters') represents the biological characteristics of the propagules, which will determine their survival probability during sampling, transport and after release into the new geographic area. The green shaded oval ('Environmental filter') represents the biotic and abiotic characteristics of the environmental parameters of the introduction area. Hypotheses' acronyms: PP Propagule Pressure, FR Fluctuating Resources, LH Life History, ER Enemy Release, PhP Phenotypic Plasticity, NA, New Associations, NW Novel Weapons, EICA Evolution of Increased Competitive Ability

The Propagule Pressure Hypothesis, also known as introduction effort, combines the number of propagules introduced and the number of introduction events. It is recognized as a major determinant of establishment and further colonization success. Indeed, high propagule pressure increases the probability of persistence of the alien species through increased genetic diversity, and thus adaptive potential, but also helps to counteract negative effects associated with small population such as Allee effects. High propagule pressure is sometimes considered as a pre-requisite of invasions rather than a driver of invasion success (Lockwood et al., 2005).

The Life History hypothesis (described as 'Ideal Weed' in plant ecology) is directly related to the invasiveness concept, and focuses on traits of the alien species enhancing competitive abilities allowing to outcompete indigenous species (Elton, 1958; Baker & Stebbins, 1965; Burns, 2008).

The Fluctuating Resource Hypothesis assumes that invasion success can be favoured if some resources are not (much) used (unsaturated niche, low to absence of functional redundancy), or if there is a temporal or spatial increase in resource availability for an opportunistic invader, either by increase of supply (eutrophication, facilitating effect of another introduced species = Invasional

Meltdown; Simberloff & von Holle, 1999) or decrease of resource use through, for example, the local extinction of a competitor (Sher & Hyatt, 1999; Davis et al., 2000).

The Enemy Release Hypothesis is probably the most famous and the most straightforward hypothesis of invasion success. Alien species released into a new environment are (partly) freed from their native natural enemies that limit their population growth or they have to defend against, leading to reallocation of resources to growth and reproduction or adaptation, increasing competitive abilities (Keane & Crawley, 2002; Colautti et al., 2004). This hypothesis is the root of many enemy-related hypotheses, but also to the Evolution of Increased Competitive Ability Hypothesis (Handley et al., 2008).

The New Associations Hypothesis highlights the potential of interspecific interactions between the alien species and the native community to influence the invasion success, these new interaction being either positive (mutualist species) or negative (new enemy – Callaway & Ridenour, 2004; Colautti et al., 2004).

The Novel Weapons hypothesis is designed for plants, and stipulates that invasive species able to produce allelopatic chemicals the indigenous competitors would not be co-adapted with would be favoured by higher competitive abilities (Callaway & Ridenour, 2004).

The ecological continuum ending in the invasive success is likely to result from the combination of these interrelated hypotheses (van Kleunen et al. 2010). In addition, the relative importance of each of these hypotheses (and many others) may vary according to the constantly evolving interaction 'species' × 'environmental characteristics', with invasive species benefiting from the current climatic changes. Meanwhile, the synergistic effect of climate change and biological invasions on multispecies interactions remains largely unexplored (Walther et al., 2009).

d) *Contributions to the understanding of the invasion process from evolutionary biology*

Biological invasions constitute natural experiments not only for ecologists, but also for evolutionary biologists: natural colonisation processes occur at ecological timescales, instead of geological ones, and as such can capture on-time processes leading to the adaptation of invasive species to novel environments and novel selective pressures. Despite early suggestions placing evolutionary adaptation as a main explanation for invasiveness (Baker, 1974), invasive species have not been studied as long in evolution as in ecology (Lee, 2002; Prentis et al., 2008). Nonetheless, key achievements have been made in our understanding of evolutionary changes occurring during invasion. Since the beginning of the 2000's, the development and lower expenses of molecular analyses techniques, have greatly contributed to the development of investigations of non-model species, which is often the case for invasive organisms (Lee, 2002; Blanchet, 2012; Bock et al., 2015).

Molecular tools have been extensively used for the reconstruction, through phylogeography, of invasion routes. Such studies are conducted in order to define the native origin and range of the

species with quite precision, and to assess transport vectors (Estoup & Guillemaud, 2010; Cristescu, 2015). Associated with historical knowledge (if available), these techniques constitute a powerful tool enabling to better understand invasive populations' origins, and allow subsequent comparisons between source and introduced populations to further test eco-evolutionary hypotheses.

Invasive populations are generally thought to be founded by a small number of individuals (Dlugosch & Parker, 2008), representing a more or less reduced fraction of the pool of genetic diversity of the source population (Nei et al., 1975; Barrett et al., 1990), and probably high inbreeding levels in introduced populations (van Buskirk & Willi, 2006). Genetic diversity is the basis of the adaptive potential of populations to adapt to new environmental conditions (Fisher, 1930). From these two assumptions emerges the Genetic Paradox of Invasions (GPI): how invasive populations manage to adapt to the novel selective pressures encountered in the introduced area despite reduced genetic diversity (Sax & Brown, 2000)? In this context, genetic analyses of invasive populations first focused on comparing levels of genetic diversity between native and introduced populations (Lee, 2002; Cox, 2004; Bossdorf et al., 2005; Novak & Mack, 2005; Wares et al., 2005; Dlugosch & Parker, 2008) and found contrasted outcomes. Many authors showed decreased levels of genetic diversity in the introduced population compared with the native populations, and cases with increased genetic variation in the invasive population were likely due to admixture (multiple introductions) from different source populations (Novak & Mack, 1993; Genton et al., 2005). Genetic bottlenecks, however, do not seem to constrain invasive success (Sax et al., 2007), or even prevent rapid adaptive change (Prentis et al., 2008), as illustrated with the invasion case of *Drosophila subobscura* in Chile (Huey et al., 2005). Prentis et al. (2008) pointed out that even though genetic bottlenecks could enable rapid adaptive change to occur (through the conversion of epistatic variance to additive variance for example [Whitlock et al., 1995] or a complex interaction between inbreeding depression and a benign introduced environment [Schrieber & Lachmuth, 2016]) under special conditions, the genetic depletion of introduced population would generally impede fast adaptive change to occur.

In the same fashion as in ecological studies, evolutionary investigations of invasive species reported genetic features that could assist invasion success (Lee, 2002). For instance, inter- and intra-specific hybridisations could counteract genetic depletion of founder events and even create transgressive phenotypes (Rieseberg et al., 2003), or genomic rearrangements, like chromosomic inversions, could occur (Prevosti et al., 1988). A major question requiring deeper investigations concerns the substrate of genetic variation upon which natural selection could act for mounting adaptive genotypes/phenotypes in introduced populations with low additive genetic diversity. So far, hypotheses favour standing genetic variation over new mutations, because of the lower probability of the latest to happen, to be beneficial and to reach fixation (Bock et al., 2015).

An alternative (but not mutually exclusive) hypothesis to explain local adaptation of introduced species encompassing major loss of genetic diversity would be the phenotypic plasticity,

with "general purpose genotypes" (Baker, 1965) conferring high performance in a broad range of environmental conditions (Richards et al., 2006). Plasticity would theoretically be beneficial only in the early stages of invasion, because of the probable costs of maintaining plasticity (van Kleunen & Fischer, 2005) against genetic assimilation (fixed expression of the locally adapted phenotype) after establishment. However, fluctuating environments (human disturbance) are predicted to favour phenotypic plasticity, and could then explain long-term persistence of plasticity in disturbed introduced areas. Finally, molecular genetic mechanisms could also permit the maintenance of adaptive plasticity, through environmentally sensitive alleles or regulatory loci that induce differential expression patterns depending on the environment, which can also be achieved through epigenetic variations (Bock et al., 2015).

Evolutionary studies of invasive populations represent double edged investigations, with genetic and genomic tools highlighting features of invasive species explaining their success, and with the natural experiments that constitute biological invasions allowing to investigate in real time the influence of evolutionary and ecological forces on the processes of species adaptation or speciation. Fitzpatrick et al. (2011) argued the short timescales of biological invasions are a major problem in studying genetics patterns of invasive species because expanding populations, non-equilibrium dynamics and few generations violate required assumptions of classic population genetics. These authors recommended increased caution in using and interpreting results from population structure, genetic differentiation or estimated migration rates, because mutation-drift equilibrium and patterns of genetic variation were certainly not stable, thus not trustworthy, in these young and changing populations.

Despite investigating the same process, interest is somewhat different between evolutionary biology and ecology, as highlighted by Richards et al (2006) who contrasted the precision of molecular studies and the realism of ecological experimental designs. When combined, the two approaches could be a powerful tool to get insights into generalization patterns. For example, Estoup & Guillemaud (2010) promoted the use of both historical and genetic data to investigate introduction histories; Stinchcombe & Hoekstra (2007) proposed to combine genome scans and quantitative trait loci (QTL) mapping to investigate genomic regions and genes/traits involved in rapid adaptation of invasive species.

e) *Dispersal of range expanding specimens*

Invasive populations are characterised by range expansion, a stage for which dispersal ability represents a prominent aspect. The capacity of individuals to disperse determines the speed at which colonisation of new habitats will occur. Unlike demographic expansion, sequential founder events constitute the range expansion process (Slatkin & Excoffier, 2012). As such, ecological hypotheses explaining range expansion and its dynamics are partly based on a combination of short- (stepping stone) and long-distance jumps (Kot et al., 1996). The few individuals colonizing new areas, more and

more distant from the core population, should have direct fitness advantages. Indeed, recently established specimens should benefit from decreased intra-specific competition pressure (Travis & Dytham, 2002; Burton et al., 2010). Moreover, these founder individuals at the invasion front, supposedly characterized by a majority of individuals with good dispersal abilities, will share and transmit their genetic background (assortative mating). As this phenomenon repeats as the invasion front moves forward, dispersal traits should be enhanced at the leading edge of the range expansion, generating phenotypic differentiation between front and core individuals. Behind the front wave and in core populations, higher population densities should favour competitive abilities of individuals, rather than biological traits enhancing their dispersal capacities. The promotion of dispersal traits at the invasion front has been highlighted in the cane toad rapidly invading the north-east coast of Australia (Phillips et al., 2006, 2010), and this has led to the theory of spatial sorting (Shine et al., 2011).

Consequences of range expansion for population dynamics and potential of invasive species to encompass rapid range expansion and adaptation are now being extensively studied in theoretical and empirical works (Simmons & Thomas, 2004; Hughes et al., 2007; Léotard et al., 2009; Monty & Mahy, 2010; Sultan et al., 2013; White et al., 2013). Yet, our current understanding of the processes generating pheno- or genotypic variation in dispersal ability along the invasion gradient remains incomplete, especially under non-equilibrium conditions like range expansion and invasion. Moreover, human-assisted dispersal events and multiple reintroductions impede our understanding of the actual expansion sequence. While theories suggest that spatial sorting may contribute selecting dispersive phenotypes at the front, the magnitude of this process is expected to be impeded by a range of life history trade-offs that may either facilitate or constrain the species' invasion success, with subsequent fitness consequences (*i.e.* constrained energetic outputs). This area of biological invasion seems very promising, and also challenging; for example, genome scans techniques traditionally allow to discriminate evolutionary forces occurring on the genome (demographic events or genetic drift impact the whole genome, selection acts on particular loci). But in the context of range expansion, drift is expected to be strong in the low density front populations, allowing random alleles (potentially deleterious) to arise from standing variation and reach high frequencies, thus to displaying a positive selection-like signal (Klopfstein et al., 2006). White et al. (2013) showed this problem could be solved by sampling replicated invasion front populations, as surfing produced by drift would result of independent allele 'selection', when adaptive trait promotion would impact the same loci.

The phenotypic differences generated by spatial sorting could help understand the genetic mechanisms underlying dispersal evolution (Handley et al., 2011), as well as eco-evolutionary trade-offs between functional traits such as dispersal, reproduction, growth. Understanding the dynamics and mechanistic of range expanding invasive populations would also be useful in other context, such as populations range shifts in response to climate change or introduction of biological control (White et al., 2013).

2 - Presentation of the studied system: a ground beetle invading the French subantarctic Kerguelen Island

a) *The subantarctic region, geographic context*

Subantarctic islands are located between 45°S and 54°S, close to the Antarctic Polar Front Zone, and are the main representatives of Southern hemisphere's terrestrial ecosystems at mid- and high-latitudes (Bergstrom & Chown, 1999). Subantarctic islands include South Georgia in the South Atlantic region, Prince Edward, Crozet, Kerguelen and Heard archipelagos in the South Indian region, Macquarie Island and the New Zealand subantarctic islands (Campbell, Auckland, Antipodes, Snares and Bounty islands) in the South Pacific region. They are often combined with the islands of the cold temperate zone (the Falkland archipelago, Tristan da Cunha archipelago and Gough Island in the South Atlantic, Saint-Paul and Amsterdam islands in the South Indian ocean), altogether forming the Southern Ocean Islands (SOI). For clarity purpose, the following content of this part will focus on subantarctic islands strictly.

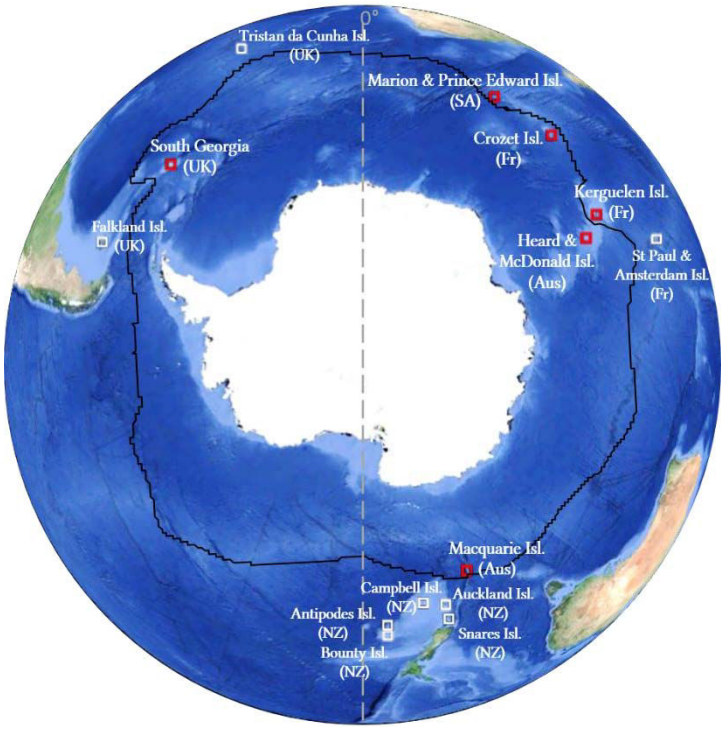

Fig. 3 Localization of Southern Ocean Islands. Subantarctic islands are squared in red, whereas cold temperate islands are squared in white

b) *Differences and similarities of subantarctic islands*

In many aspects, these isolated landmasses are very different from each other. First, the area of the islands highly varies, from 1.35 km² for Bounty Island to 7200 km² for the Kerguelen Islands, as well as their isolation from other islands (if they are associated in archipelagos for example) and from continental landmasses. Second, even if most of these islands are of volcanic origin (except for South Georgia that share continental origins with the southern tip of South America) age of these oceanic islands scales from *ca.* 39 million years for Kerguelen Islands to 79,000 years for McDonald Island (curiously these two extremes belong to the same oceanic plateau). Glaciation extents are currently

much reduced as compared with the last glacial maximum (26.5 and 19 ka; Clark et al., 2009), and only South Georgia and Heard Islands remain heavily glaciated, with ice-sheets covering respectively 57% and 80% of their total areas (see Tab. 1 for an overview of the main particularities of these subantarctic archipelagos, based on Chown et al., 1998; Chown & Convey, 2016).

Tab. 1 Summary of geographical, physical, ecological and historical variables of subantarctic islands and cold temperate islands. Islands of the same archipelago are connected by brackets

Island	Localisation	Area (km²)	Max altitude (m)	Age (myr)	Distance to continent (km)	Mean annual temperature (°C)	Nb of humans	Vascular plants	Insects	Land birds	Sea birds	Mammals
Macquarie	54.62S, 158.9E	128	433	11.5	990	5.13	57	40 / 5	24 / 7	0 / 4	23	4
South Georgia	54.25S, 37.0W	3755	2950	120	2210	1.49	111	25 / 53	21 / 8	2 / 1	26	3
Heard	53.1S, 73.5E	368	2745	20	4570	1.74	0	10 / 1	11 / 1	1 / 0	18	0
McDonald	53.03S, 72.6E	2.6	230	0.079	5000	1.85	0	5 / 0	6 / 0	1 / 0	9	0
Campbell	52.5S, 169.17E	113	567	16	700	7.83	7	140 / 88	176 / 19	4 / 10	22	2
West Falkland	51.5S, 60.5W	3500	701	2500	530	7.67	101	153 / 66	78 / 5	38 / 4	24	11
East Falkland	51.5S, 58.5W	5000	705	2500	550	7.29	**2701**	149 / 78	132 / 22	38 / 4	24	11
Auckland	50.83S, 166.0E	626	668	18	465	9.22	0	188 / 33	237 / 10	11 / 10	28	4
Antipodes	49.68S, 178.77E	21	366	0.5	872	7.9	0	68 / 2	63 / 13	4 / 4	21	1
Kerguelen	49.37S, 69.5E	7200	1840	30	4110	3.46	123	30 / 36	27 / 13	3 / 0	33	7
Snares	48.12S, 166.6E	3.28	152	120	209	10.95	0	20 / 2	137 / 8	6 / 4	17	0
Bounty	47.72S, 179.0E	1.35	89	189	624	9.63	0	0 / 0	10 / 0	0 / 0	11	0
Marion	46.9S, 36.75E	290	1230	0.45	1900	5.53	51	23 / 17	19 / 16	1 / 0	27	2
Prince Edward	46.63S, 37.95E	44	672	0.21	1900	5.59	0	21 / 2	18 / 3	1 / 0	28	0
Pingouins	46.5S, 50.4E	3.16	360	1.1	2740	4.87	0	13 / 1	24 / 0	2 / 0	22	0
Est	46.43S, 52.2E	130	1090	8.75	2740	4.75	0	19 / 5	35 / 2	2 / 0	33	1
Possession	46.42S, 51.63E	150	934	8.1	2740	4.78	49	19 / 101	44 / 7	2 / 0	33	1
Cochons	46.1S, 50.23E	70	775	0.4	2740	4.87	0	18 / 6	29 / 3	2 / 0	18	3
Apôtres	45.97S, 50.43E	3	289	5.5	2740	5.11	0	13 / 2	13 / 0	2 / 0	23	0
Gough	40.33S, 9.54W	57	910	6	2670	12.35	38	57 / 24	29 / 15	2 / 0	20	1
St Paul	38.72S, 77.53E	8.1	268	0.5	3000	14.59	0	9 / 10	13 / 9	0 / 1	10	3
Amsterdam	37.83S, 77.52E	55	881	0.7	3000	15.17	38	26 / 81	19 / 18	0 / 3	14	4
Nightingale	37.42S, 12.5W	4	400	18	2820	15.32	0	34 / 6	34 / 4	3 / 0	14	0
Inaccessible	37.25S, 12.75W	12	600	6	2820	15.32	0	55 / 20	38 / 12	4 / 0	17	0
Tristan de Cunha	37.1S, 12.25W	86	2060	1	2820	15.32	**315**	64 / 93	38 / 37	1 / 1	19	8

Despite those differences, subantarctic islands are often considered as a biogeographical region because of their similarities in terms of climatic conditions and biota composition. Their climate is under strong oceanic influence, and is windy, cloudy, and characterized by high precipitation levels and low seasonal variability. The mean annual temperature ranges from 1.5 to 10°C (Chown et al., 1998). Species richness and functional diversities are low on these scattered terrestrial ecosystems. Subantarctic islands' biodiversity has been considered anomalous and disharmonic because of the underrepresentation of orders or taxa that are common elsewhere, and because of the reduced functional diversity. Flora is dominated by shrubs, grasses and cushion plants. There are no indigenous terrestrial vertebrates except for three species of ducks, one passerine endemic of South Georgia and two species of sheathbills, but coastal environments are crucial breeding and moulting sites for several charismatic species of seals (*Mirounga leonina*, *Arctocephalus gazella*) and sea birds (*Diomedea exulans*, *Macronectes giganteus*, *Aptenodytes patagonicus*). The terrestrial fauna is dominated by arthropod communities, the more represented groups being springtails, mites, flies, weevils and beetles. Most of these species are decomposers or saprophagous, few are herbivores and predation is thought to be insignificant (Convey & Lebouvier, 2009). Subantarctic islands' fauna host higher proportions of flightless or wingless insect taxa compared with other habitats (insular or continental, Gressitt & Weber, 1959; Ewing, 2009; Laparie et al., 2016), and this situation was suggested to stem from the cold and windy environments at these high latitudes (Roff, 1990). Taxonomists are still exploring underexamined groups such as bryophytes, lichens or diatoms, and close to nothing is known regarding microfaunal, microbial or viral diversities (but see Frenot et al., 2005).

c) *Human presence and impacts*

Subantarctic islands have no permanent inhabitants (continuous presence of human cannot exceed 16 months nowadays) and have a very recent human history as compared with other insular ecosystems. Indeed, the discovery of the subantarctic islands ranges between late 16th century and the 1850's. Subantarctic islands were ground for sealing and whaling during the 19th century, which led to the near extinction of fur and elephant seals, and more or less temporary coastal human settlements. More permanent human occupation occurred since the first half of the 20th century with the building of research stations on Marion Island, La Possession Island (Crozet), Kerguelen Islands and Macquarie Island. Among the other subantarctic islands, some of them, or parts of them, are temporarily visited by scientists for summer campaigns, and others (or other parts of them) are natural reserves without any visitor (the west coast of the Rallier du Baty peninsula at the Kerguelen Islands, Cochons island at the Crozet archipelago for example). Despite the extreme geographic isolation of the subantarctic islands, and the recent human occupation, many plant and animal species were introduced. Even if there were voluntarily introductions, in attempts of economical enhancements (grazing cattle, reindeers), several species were introduced accidentally in imported material and food supplies (Poacea and Asteracea species, aphids, pooflies). Many introduced species are cosmopolitan or of European origin (Slabber & Chown, 2002; Frenot et al., 2005).

Chown et al. (1998) found a positive correlation between species richness of introduced taxa with area and temperature of southern ocean islands, and they also reported that the number of visitor per year (scientists) covaried positively with island area. The authors suggested that larger islands have more introduced species because of the increased human load (vectors), and that warmer islands increased the chances for propagules to establish viable populations. Climate warming and increasing proportion of tourism at the subantarctic islands are likely to facilitate the arrival and establishment of new species in the region. Tourists mostly have new equipment when they arrive, but this is not the case for most scientists (Chown et al., 2012), and ultimately the material and food discharged on islands represent the biggest risk of introduction of exotic species.

At a more global scale, humans also have indirect impacts on subantarctic islands through climate change. Long-term monitoring (> 50 years) of meteorological conditions on Kerguelen, Marion and Macquarie islands revealed increased of mean annual air temperature higher than 1 °C, drastic decrease in precipitation level, decrease in the total number of freezing days at the Kerguelen Islands and increased number of annual hours of sunshine at Macquarie Island (Smith, 2002; Frenot et al., 2005; Lebouvier et al., 2011). This rapid change may have profound impacts on natural ecosystems, in terms of communities and functionality dynamics. For example, Chown & Smith (1993) investigated the interaction between climate change and the introduced house mouse population altering trophic web on Marion Island (also see Table 5 in Frenot et al., 2005 for a synthesis on the potential interactions between climate change and biotic interactions).

d) *The Kerguelen archipelago*

The Kerguelen archipelago is the oldest of all of the subantarctic islands of volcanic origin (Chown et al., 1998). It also is the biggest, with a total area of 7200 km², unevenly divided into the main island, which represents 92% of the total area, and surrounded by approximately 300 islets. The eastern part of the main island is covered by the 400km² [and shrinking] Cook glacier. This archipelago was discovered in 1779 by Yves Joseph de Kerguelen de Trémarec, and the first seasonal and local human settlements occurred during the whaling period (from the second half of the 19th century to the inter-war period). In parallel (1910 - 1930), the Bossière brothers tried to raise sheep but their three attempts failed, living at Port Couvreux the remaining of the old farm and numerous introduced grasses. The scientific station of Port-aux-Français was built in 1951, and, since then, human presence is more permanent. With a carrying capacity of 120 persons during the austral summer and 45 during the austral winter, Port-aux-Français is the most populated research station of all of the subantarctic islands. The RV Marion Dufresne, the principal way to reach the island, supply the station four times per year.

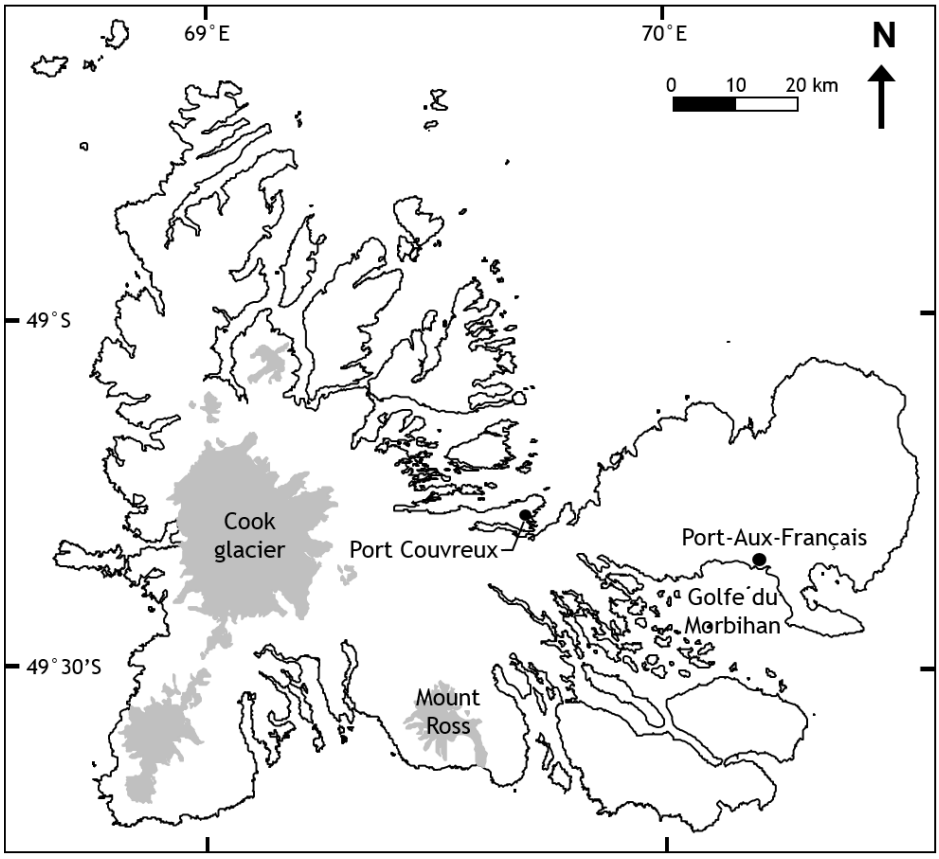

Fig. 4 The Kerguelen Archipelago

e) *Relevance of subantarctic islands in ecology*

The subantarctic islands are considered as open-top laboratories that allow to study ecological processes from molecular to biogeographical levels. Compared with terrestrial ecosystems from other geographic regions, these islands host reduced specific and functional diversities, thus facilitating studies assessing interspecific interactions such as trophic web structure, intra-guild competition (Davies, 1987; Chown & Smith, 1993; Todd, 1996), or functional ecology (Crafford, 1990; Slabber & Chown, 2002). Detection and monitoring of introduced species is also facilitated by reduced biodiversity (there are examples of transient alien species; Pugh, 1994; Gremmen & Smith, 1999; Frenot et al., 2005; Chown et al., 2008), moderate to small size of the terrestrial areas and the spatially limited human settlements (harbours and research stations are the main entrance points of material and personal, and this is likely the case for alien species, as they are numerous in the vicinities of research stations; Frenot et al., 2005). Apart from undiscussed advantage in terms of conservation, this allows to investigate mechanisms and dynamics of biological invasions such as range expansion, impacts of new predators or competitors on native biota, post-introduction population modifications (Pontier et al., 2002; Laparie et al., 2010, 2013; Renaud et al., 2013). Human history in these ecosystems is recent, but anthropogenic are significantly increasing (Convey & Lebouvier, 2009), the major and direct human-associated environmental modification being species introductions. Conservation politics led to several eradication trials on different islands, which in turn led to long-term survey of

recolonization processes and resilience assessments of native communities (Chapuis et al., 2004; Lebouvier et al., 2011).

These islands are major places to investigate the actual ecological challenge in understanding the combined effects of the interaction between biological invasions and climate change on biotas.

Even if any region and ecosystem can host non-native (alien) species, habitats from oceanic islands are often described as being more susceptible to biological invasions than those from mainland. The strong geographical isolation of oceanic islands originating from deep sea volcanic activities, together with the absence of many functional groups that are present elsewhere, make them highly vulnerable to several components of environmental perturbations, including climate changes and biological invasions. This is even truer for the southern oceanic islands. As example, the French Kerguelen Islands only support 23 native insect species, 22 native vascular plant species, and no native terrestrial vertebrates, except two non-marine birds.

f) Merizodus soledadinus*: Taxonomy and distribution*

Merizodus soledadinus Guérin-Meneville 1830 (Coleoptera:Carabidae) is a wingless generalist predator ground beetle belonging to the subtribe Trechitae, tribe Zolini (the phylogeny of the Trechitae was unresolved until investigations on adult and larva morphology and 16S sequences that confirmed the monophyly of both Trechitae and Zolini - Roig-Juñent & Cicchino, 2001; Grebennikov & Maddison, 2005). The nomenclature *Merizodus soledadinus* was given by Jeannel (1926, 1940, 1962) but Johns (1974) transferred it to *Oopterus soledadinus*, which was the species name used in the 1990's and 2000's. Since 2009 the *Merizodus* genus is used, a choice argued by the South American distribution of this species, whereas the specimens from the *Oopterus* genus are distributed in New Zealand distribution (Lalouette, 2009). *Merizodus soledadinus* has a cold temperate distribution, the native range including the southern tip of South America (Tierra del Fuego and Patagonia) and the Falkland Islands. It was accidentally introduced at the Kerguelen Islands, where it was first spotted by R. Jeannel in 1939 in the rubbles of the former ship farm of Port Couvreux (Jeannel, 1940), and in South Georgia, where it was first spotted in 1963 in the surroundings of the whaling station of Grytviken (Darlington, 1970).

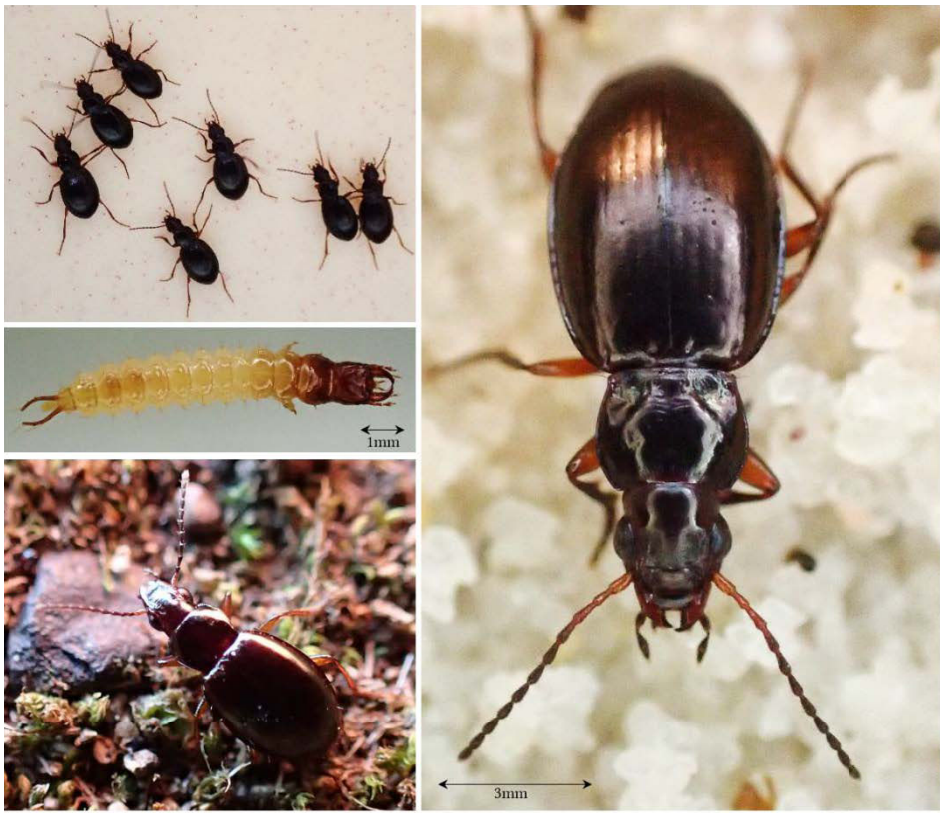

Fig. 5 *M. soledadinus* illustrations

g) *Our current knowledge of the biology of* M. soledadinus

Previous studies on this species were carried out on specimens from the two introduced islands only, and are essential for our understanding of the main characteristics of the autecology of *Merizodus soledadinus* in the subantarctic region. Specifically, studies were conducted to enhance our knowledge of its life cycle (Ernsting, 1993), geographic distribution and range expansion (Dreux et al., 1992; Todd, 1996; Chevrier et al., 1997; Brandjes et al., 1999; Convey et al., 2011; Renault, 2011), physiological responses to a variety of biotic or abiotic factors (Todd & Block, 1997; Laparie et al., 2012; Siaussat et al., 2012; Hidalgo et al., 2013), plasticity to thermal variations (Todd, 1997; Lalouette et al., 2012; Laparie & Renault, 2016), morphometric changes (Laparie et al., 2010, 2013), and habitat occupation (Renault et al., 2015). This species is considered invasive at both South Georgia and Kerguelen Islands, where it exhibits since the past decades rapid demographic and range expansion, with deep impact on indigenous/endemic arthropod communities that evolved without significant predation pressure. For instance, at the Kerguelen Islands, the establishment of *M. soledadinus* into new habitats is correlated with decreased abundance and local extinctions of native dipteran species (Frenot et al, 2005; Lebouvier et al, 2011). In South Georgia, the ecological impact differs as compared with the Kerguelen Islands, as this island also hosts another introduced predatory ground beetle, *Trechisibus antarcticus*, very similar in size and morphology to *M. soledadinus*. Convey et al. (2011) showed recent and rapid range expansion of these two exotic insect species, and their co-occurrence in some localities. Yet, *T. antarcticus* seems to have better competitive abilities than *M. soledadinus*, as depicted by higher

individual densities and wider geographical spread at South Georgia, even though the first record of *T. antarcticus* on this island is posterior (1982, Brandjes et al, 1999).

The invasion case of *M. soledadinus* at the Kerguelen Islands is characterized by several particularities as compared with other invasion cases. First, the history of the introduction of *M. soledadinus* in this archipelago is well documented: thanks to the remote position of the Kerguelen Islands, far away from commercial sailing routes, together with the recent human history and records of ship discharged material and animals, it allowed to retrace the most probably unique date of introduction of *M. soledadinus* at Port Couvreux. This introduction event most probably occurred during the unloading of sheep and hay cargo from the Falkland Islands in august 1913. Later, in 1939, René Jeannel only found *M. soledadinus* at Port Couvreux (a lag time between introduction and spread of at least 26 years), but in high abundances (stage IVb of the Colautti & MacIsaac [2004] framework).

Second, the range expansion was long-term monitored on the eastern half of the main island of the Kerguelen Islands, and on the islets of the Golfe du Morbihan since 1974. Maps of the chronosequence of *M. soledadinus*' range expansion are presented in the Fig. 6. These observations allow to estimate (more or less precisely for older sites) the period of colonization of most localities by *M. soledadinus*, and thus the residence time of the populations. This residence time was found to be negatively correlated with the abundance of indigenous dipteran species, especially *Anatalanta aptera* Eaton, 1875.

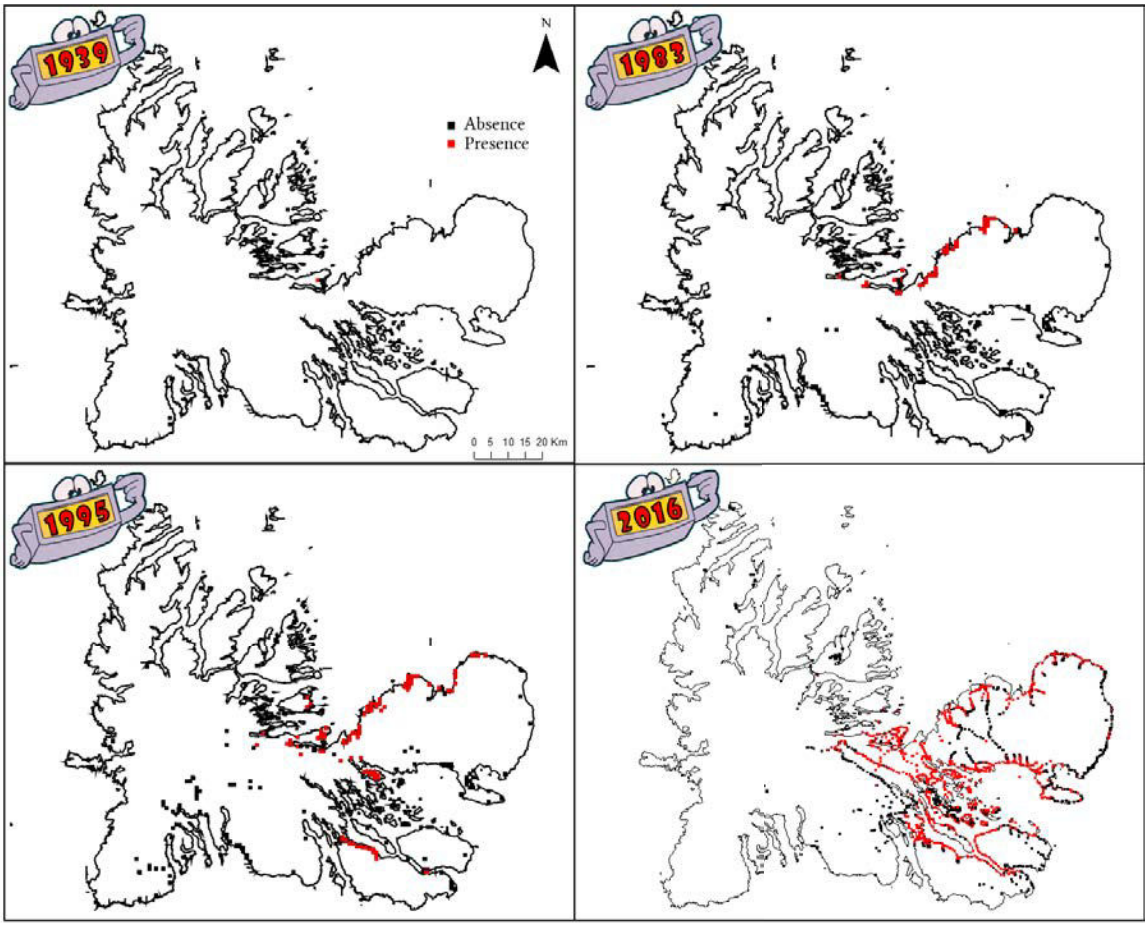

Fig. 6 Chronosequence of the range expansion of *M. soledadinus* at the Kerguelen Islands.

Third, a recent study highlighted a quantitative relationship between residence time and morphology in this invader, as illustrated in the Fig. 7 (Laparie et al. 2013). Like the cane toad, this pattern could be due to spatial selection and increased dispersal abilities at the invasion front.

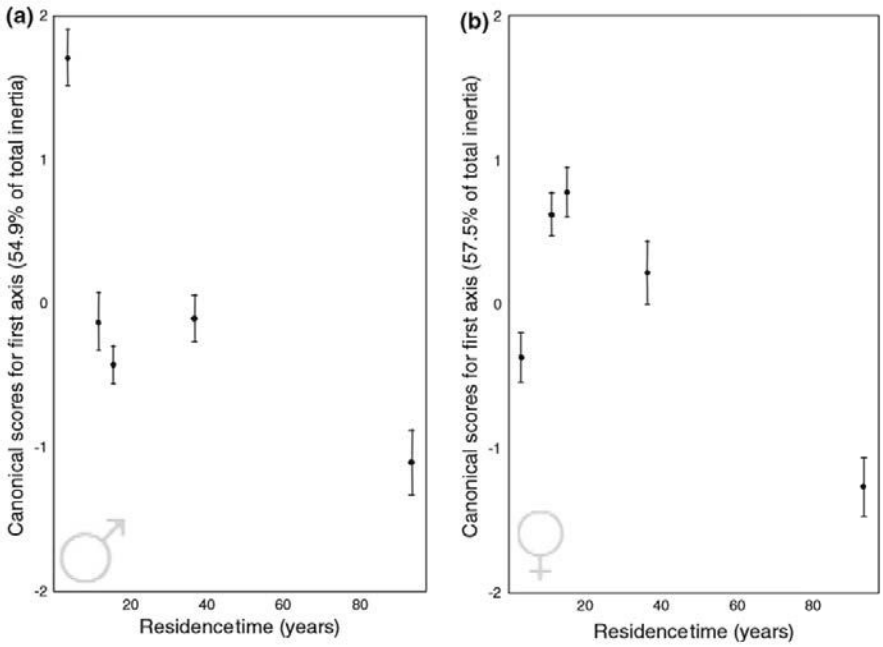

Fig. 7 Morphological variations between five populations characterised by increasing residence time, according to the scores of individuals along the first axis of a multivariate analysis (CDA) including seven traits measured on 146 males (a) and 150 females (b)(modified from Laparie et al., 2013)

This putative progressive selection on dispersal power through the 103 years-long invasion succession thereby sets a fertile ground to further investigate this evolutionary process.

3 - Objectives and structure of the present dissertation

More insight is needed into the main eco-evolutionary mechanisms that assist biological invasions. Ecological knowledge on invasive species and invaded environments are fundamental to understand invasion history and determinants of the invasion success. We conducted multi-scale ecological exploration of an invasion phenomenon to fulfil this goal, focusing on the case of the invasion of the Kerguelen Islands by the predatory ground beetle *M. soledadinus*. As previously outlined, the introduction and subsequent spread of this insect over the Kerguelen archipelago presents a rare combination of valuable features that facilitates investigations on various aspects of an invasion process. Particularly, with this model system it is possible to highlight mechanisms or processes that are blurred or hindered in other invasions cases due to complex biotic interactions, genetic admixture or anthropogenic disturbance of the invaded communities and habitats.

Rooted into the context of biological invasions, the aim of this Ph.D thesis is to contribute fitting together pieces of the ecological puzzle of the invasion success story of the ground beetle *M. soledadinus*. The major aim of the work was to improve our knowledge of this insect species at the Kerguelen Islands (autecology) and to provide new insights that would increase our understanding of its invasion success. During this work, different methodologies were used to attain the objectives,

encompassing classical autecology, genomics, metabolomics stress physiology and behaviour. Specifically, the following interrogations are raised:

- Do individuals of *M. soledadinus* from the Kerguelen Islands show reduced levels of genetic diversity compared with their counterparts from native areas? This expectation results from the fact that the single and unique introduction event has probably resulted in a drastic founder event. To what extend genetic diversity is shared between invasive populations from South Georgia and those from the Kerguelen Islands? Can we see a genetic structure along the invasion gradient of *M. soledadinus* on the main island of the Kerguelen archipelago?
- Do specimens from front and core population differ in terms of dispersal-related physiological and behavioural traits? In other words, is there a phenotypic differentiation among insects from populations of different residence time?
- What are the main factors driving habitat suitability (from *M. soledadinus* point of view), particularly in terms of thermal conditions of the potential invaded habitats, water and food availability? Is the recent colonisation of altitudinal habitats by *M. soledadinus* assisted by the ongoing climatic changes occurring in the subantarctic region?
- What are the main ecological and biological characteristics of *M. soledadinus*? For instance, what is the lifespan of adults? Can we estimate females' fecundity? Does adults show seasonal variations in terms of activity or body reserve compounds (*i.e.* what happens during the cold season)?

The different studies described in the following chapters have been designed in order to try to answer those questions.

An initial, but primordial investigation is presented in the Chapter 1. This work aimed at providing new insights into the biogeography of this species, by comparing population genetics of specimens of *M. soledadinus* from the Kerguelen Islands with other known representatives of this insect species, and to further compare the obtained genetic information with the historical data available for this insect. At a different scale, population genetics of specimens from the Kerguelen Islands sampled from populations along the invasion gradient are also unravelled as a first step allowing a better understanding of population dynamics and adaptive potential.

The second chapter focuses on the characterisation of the phenotypes of individuals sampled from different populations along the invasion gradient. We were interested in examining if the gradient of increased morphology accompanying expansion range was associated to changes in other life history traits, to investigate hypothesis on expansion-associated phenotypic modifications.

Habitat requirements of adults *M. soledadinus* are investigated in chapter 3, through experimental exposition of individuals to desiccating conditions. These abiotic conditions could have an effect on individual dispersal and constitute a significant ecological barrier to its geographic expansion.

The fourth chapter highlights new features on the autecology of *M. soledadinus*, through investigations of seasonal variations in major life history traits, in order to better understand ecological requirements and population dynamics.

Finally, the fifth chapter presents the phenotypic characteristics of individuals sampled along altitudinal gradients. In the context of climate change, we suggest that colonisation of altitudinal habitats should be facilitated, as climatic changes may alter abiotic and biotic environmental conditions, so that altitudinal habitats are progressively becoming suitable for *M. soledadinus*.

Parts of these studies were conducted in the field at the Kerguelen Islands (two stays of 3 months each during this three-year Ph.D), and under controlled conditions at the research station of Port-aux-français. This field work was supported by the French Polar Institute *via* the framework of the programme IPEV 136. Other parts of this work were carried out at Rennes, and genetic studies were conducted at the Royal Belgian institute of natural Sciences, Brussels (three stays of 1 months each). At Rennes and Brussesl, experiments were funded by the grand InEE-CNRS Enviromics 'ALIENS' and by SYNTHESYS.

Chapter 1

New insights into the ecology of *Merizodus soledadinus*, a predatory carabid beetle invading the sub-Antarctic Kerguelen Islands

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Highlights

- Knowledge is lacking still on main life history traits of *M. soledadinus*, including lifespan, fecundity, activity in the cold season, despite being fundamental insights into population dynamics
- Adult longevity under controlled conditions was determined, seasonal activity was monitored through pitfall trapping, and temporal variations of egg load and body reserves were analysed in field-collected individuals.
- Maximum adult life-span reached 710 days, half individuals surviving around 240 days. Females carry eggs evenly during the year (8.94 ± 3.56 eggs per female), and it was not possible to pinpoint any clear egg-laying period.
- Results demonstrated the year round activity of this predator, body stores (glycogen and proteins) did not vary significantly over the year, suggesting that adults continuously feed

1 - Introduction

Despite the prominent ecological and socio-economic impacts of non-native species (Pimentel et al., 2005; Vilà et al., 2010), our knowledge of the main determinants associated with invasion success is still incomplete (Pyšek & Richardson, 2008; Gurevitch et al., 2011). Not all non-native species become invasive following their introduction into new geographical areas (terminology defined in Falk-Petersen et al. [2006]), likely because of the diversity of interconnected biotic and abiotic parameters that drive invasion success (Williamson, 2006). In parallel, several theories, including the evolution of increased competitive ability, the novel weapon hypothesis or the enemy release hypothesis have been formulated over the past decades to try explaining how non-native species successfully overcome novel environmental filters (Callaway & Ridenour, 2004; Colautti et al., 2004; Handley et al., 2008; van Kleunen et al., 2010). Invasiveness has also been suggested as a pivotal determinant of invasion rate (Williamson & Fitter, 1996; Kolar & Lodge, 2001), and the plasticity of traits, such as fecundity, longevity, together with dispersal capability and stress resistance are of tremendous importance for the successful establishment and subsequent expansion of non-native organisms. However, there are often large shortcomings in our understanding of the biology and autecology of non-native species in introduced areas, thus impairing the understanding of invasion process.

At the sub-Antarctic Kerguelen Islands, located in the south Indian Ocean (48°30'–50°S, 68°27'–70°35'E) and subject to cold oceanic climate, multiple insect species have been accidentally introduced to the point that the archipelago now hosts more non-native than native species of arthropods (Frenot et al., 2005; Lebouvier et al., 2011). Among the introduced insects, several have spread significantly despite extremely limited human assistance, if any (Frenot et al., 2005; Lebouvier et al., 2011). The predatory carabid beetle *Merizodus soledadinus* (Coleoptera: Trechidae) was accidentally introduced from the Falkland Islands in 1913 in a single site of the Kerguelen Islands (Port Couvreux; 49°17'04.9''S, 69°41'41''E, Fig.1), where the species was first observed in 1939 (Jeannel, 1940). Historic data and long-term monitoring of the expansion of this flightless insect has showed that it colonised localities nearby the introduction site first, then spread along the north coast of the main island, and ultimately invaded several islands of the Golfe du Morbihan (likely human- or bird-assisted introductions, or passive dispersal by flotation; see Renault [2011]). When established, this predator becomes the dominant species in the colonised habitats and generates significant ecological impacts on native fauna, particularly endemic Dipterans (Ottesen, 1990; Ernsting, 1993; Todd, 1996, 1997; Chevrier et al., 1997; Lalouette et al., 2012).

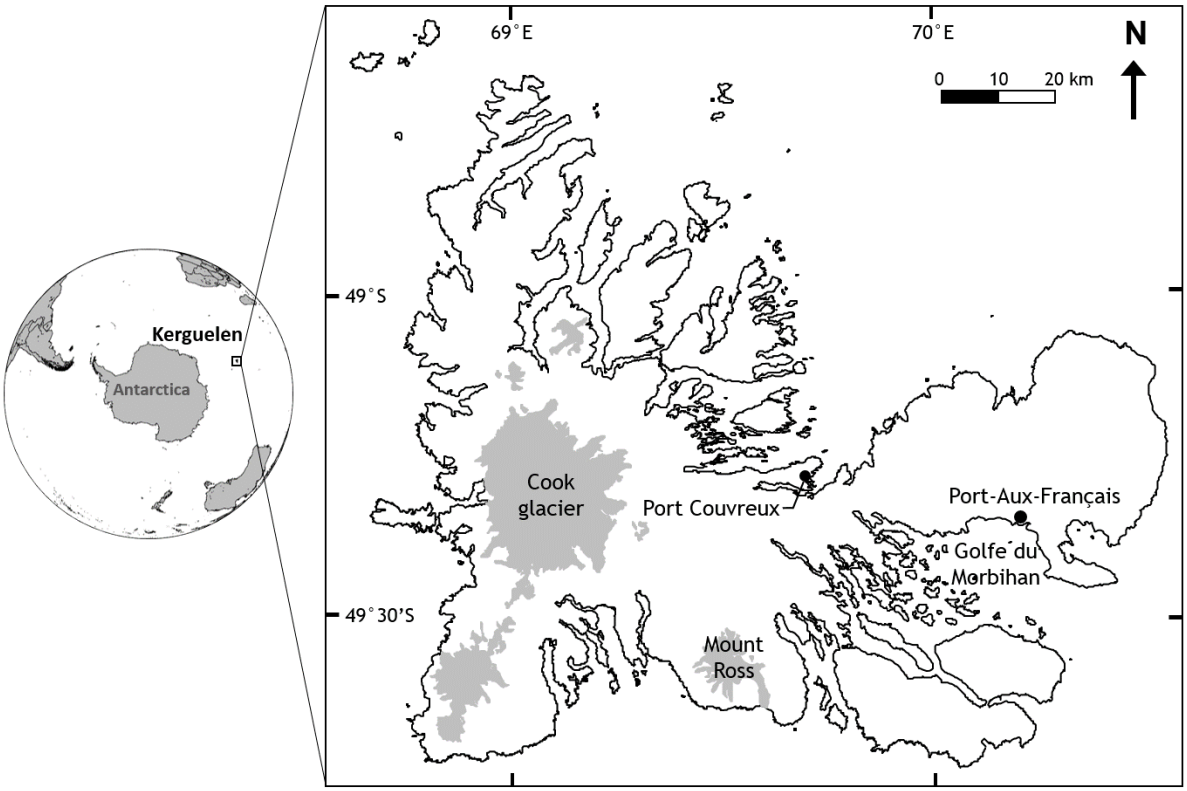

Fig. 1 Localisation of the sub-Antarctic Kerguelen archipelago. *Merizodus soledadinus* was uniquely introduced at Port Couvreux. All the experiments were conducted at the research station of Port-aux-Français, and beetles were sampled in the vicinities of the research station

Recent studies on Kerguelen Islands described the autecology of *M. soledadinus* specimens exposed to a range of environmental factors. These works reported the physiological plasticity and tolerance of this insect to thermal variations, including temperature (Lalouette et al., 2012; Laparie & Renault, 2016) and salinity (Hidalgo et al., 2013). Conversely, adults did not survive longer than two days when relative humidity decreased below 70% of relative humidity (Ouisse et al., 2016), which is consistent with the main habitats where this species thrives (Renault et al., 2015). Meanwhile, data are still missing regarding several important key elements relative to its biology (e.g. longevity, fecundity or prey preferences). The few studies conducted on this species reported by Chevrier et al. (1997) mentioned a continuous presence of adults throughout the year in the Kerguelen Islands but no published empirical data are available for these observations. In South Georgia, another sub-Antarctic island invaded by this insect (Ernsting, 1993; Brandjes et al., 1999), females egg load ranged from three to six eggs in females, and the absence of *corpora lutea* in females at the onset of summer periods (Ernsting, 1993) suggested a single breeding period.

The aim of this work is to improve our knowledge of the ecology of *M. soledadinus* at the Kerguelen Islands. To estimate adult longevity, a survival experiment was designed under controlled conditions. Based on female's reproductive status in South Georgia (Ernsting, 1993), we hypothesised an adult life span of 1-2 years. The number of eggs carried by females over the year was also investigated, since high reproductive investment can be a key characteristic in the rapid success and

establishment of invasive species (Hayes & Barry, 2008). Finally, we used long-term pitfall trapping to verify Jeannel's (1940) and later field observations that *M. soledadinus* is active throughout the year. We expected a decrease in activity during the Austral winter due to harsher climatic conditions and/or reduced prey availability, which should result into seasonal variations of protein, glycogen and triglyceride amounts, *i.e.* the major components of body fat in insects (Arrese & Soulages, 2010). Altogether, this knowledge will complement our understanding of the invasion success of this insect at the Kerguelen Islands and, in parallel, the biological limitations that may set its range edges.

2 - Material & Methods

a) *Estimation of adult longevity*

Trapping and field observations of teneral adults (individuals which cuticle is not fully melanised yet, see Online Resource Fig. ESM_1) at the Kerguelen Islands revealed that the main emergence peak occurs in late February - early March, as also observed by (Ernsting, 1993) in South Georgia. Therefore, adults of *M. soledadinus* were sampled in March 2013 under stones (ca. 5 m above sea level) around the research station (Port-aux-Français, Kerguelen Islands) to maximize the proportion of newly-emerged individuals in the samples. A total of 500 beetles were hand-collected, and used for estimating life span under controlled conditions. Soon after collection (<2h), all individuals were equally distributed into five plastic boxes (18 × 12 × 7 cm, N = 100 adults measuring 5-7 mm in body length (Laparie et al., 2010) per plastic box) lined with sterilised moist sand. Boxes were placed into a thermoregulated room (L:D 12:12h) set to 8 ± 2 °C, which correspond to thermal conditions close to those during Austral summer at this latitude (Lebouvier et al., 2011). Beetles were fed monthly with live prey, i.e. field-collected Diptera maggots (*Fucellia maritima*, *Calliphora vicina*) and enchytraeids (Annelids), both part of their natural diet (Laparie et al., 2012). The boxes were checked fortnightly, when dead beetles were counted and removed, and sand moisture adjusted as needed; the sand was changed every two months. The exact age of imagos cannot be ascertained without accurate knowledge of their emergence date. Since breeding this species under laboratory conditions has not been successful so far, the number of days elapsed since the collection date of the specimens had to be used as a proxy of life span. When present, larvae were removed from the boxes. The experiment continued until all beetles died.

b) *Annual activity in natural environments*

Sets of three pitfall traps filled with 75% ethanol were installed in 2005 at two locations (Isthme Bas 1, thereafter refer to as IS_1: -49°36'48'' S, 70°24'36.3'' E; Isthme Bas 2, thereafter refer to as IS_2: -49°36'44''S, 70°24'61.1''E) in the vicinity of Port-aux-Français (Kerguelen Islands) in order to monitor densities of arthropods active at the ground level. Over the duration of the experiment (2005-2015), all six traps were opened for five to seven days every month (twice monthly for the needs of another study in March and May 2006, December 2009, November and December 2012, February and May 2013 and October 2015).

Adults were counted in the laboratory. Since traps were not always opened for the same duration, data were standardised as 'number of individuals'/'days of operation'. Additionally, due to a high inter-annual variability in catches, annual activity was calculated as relative monthly abundance for that year.

c) *Reproductive status of females along the year*

From December 2010 to March 2012, adults of *M. soledadinus* were hand-collected every two weeks under stones (ca. 5 m above the sea level) at Port-aux-Français. They were immediately stored in 80% ethanol at -20°C until further analysis. For each sampling date, 20 females (when possible) were randomly collected for measurements of the interocular width, pronotum width and length, right elytra length, abdomen width and last abdominal sternite length (Fig. 1a). The measurements were computed from pictures taken with a video camera (AxioCam ERc 5s, ZEISS, Germany) connected to a stereomicroscope, through vectorial layouts, with AxioVision software (ZEISS, Germany). The females were then dissected to count eggs, if any (see Online Resource Tab.ESM_1 for precisions). Bigger eggs, located on the distal part of the abdomen, and smaller ones, up in the ovarioles, were distinguished (Fig. 1b & 1c).

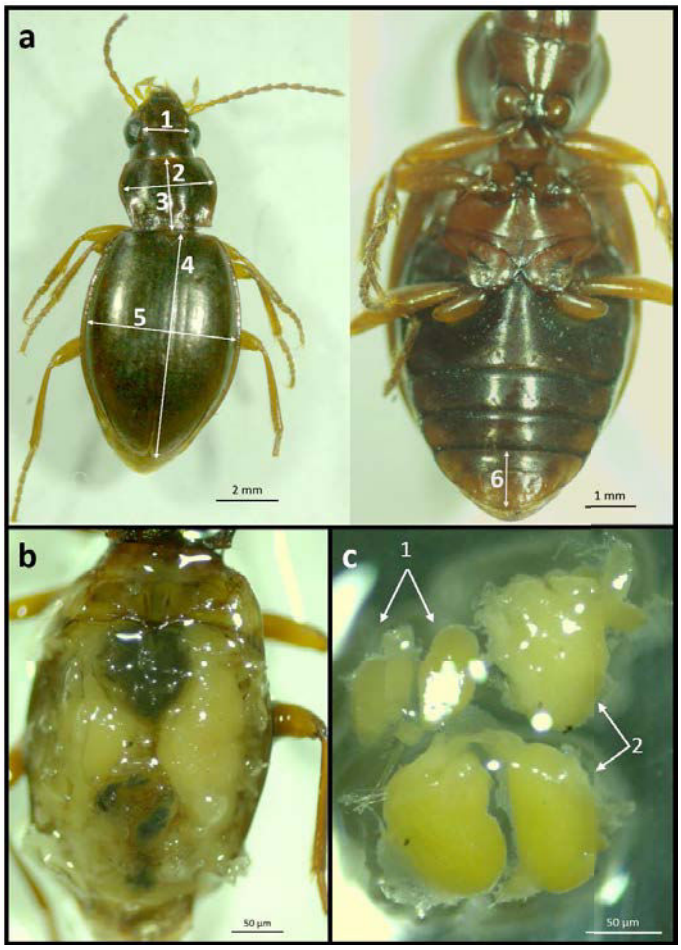

Fig. 2 Morphological traits measurements and dissection pictures of adult females of *Merizodus soledadinus*. **a**: Five morphological traits were measured on each female prior to dissection: on dorsal side interocular width (1), pronotum width (2) and length (3), right elytra length (4) and width (5) as well as last abdominal sternite length (6) on ventral side. **b**: Dissection picture of the abdomen after removal of elytra. We can see eggs in the ovaries on both sides of the abdomen. **c**: Detail of the right ovary after desolidarisation of the eggs. We distinguished 'little' eggs up in the ovaries (1) from 'big' eggs down in the ovaries (2)

d) *Annual variations of body stores*

From December 2012 to March 2014, adults of *M. soledadinus* were hand-collected under stones (ca. 5 m above the sea level) at Port-aux-Français (Kerguelen Islands). As their physiological status may vary from one day to the next depending on temperature, precipitations or trophic status of the insect, each replicate was composed of three individuals caught over three consecutive days (one individual each day) to average environmentally-induced noise. After collection, individuals were stored into 1.5 mL of 80% ethanol and stored at -20°C until analyses.

For each sampling period, six to eight replicates (each constituted of three individuals) were used to quantify total amount of proteins, glycogen and triglycerides via colorimetric assays (protocol adapted from Foray et al., [2012]). Briefly, samples were first vacuum-dried (Speed Vac Concentrator, MiVac, Genevac Ltd., Ipswich, England), and dry mass was measured (Balance XP2U Mettler Toledo, Columbus, OH, d=0.1 µg). Then, samples were redissolved in 180 µL of phosphate buffer and homogenised for 90s at 25Hz using a bead-beating device (Retsch™ MM301, Retsch GbmH, Haan, Germany). After centrifugation, 10 µL of the supernatant was used for protein assays using Bradford's technique (total protein content of each sample). The remaining 170 µL of each sample was mixed with 60 µL of 20% Na_2SO_4 and 1200 µL methanol-chloroform [ratio 2:1, volume:volume], and centrifuged (4 °C, 180g) to separate (i) total lipids (dissolved in chloroform, lower phase), (ii) carbohydrates (dissolved in methanol, upper phase) and (iii) glycogen (precipitated in the pellet). Then, 300 µL of the chloroform phase was transferred into new microtubes for triglyceride assays, and stored at -20 °C overnight. Samples were eventually vacuum-dried and redissolved in 200 µL of Triton-BSA buffer. The manufacturer's instructions were followed for the triglycerides colorimetric assay (kit reference CC02200, LTA srl, Italy). Glycogen was quantified through a reaction with 70% anthrone.

e) *Statistical analysis*

Median life span (LT_{50}) and 90% of maximum life span (LT_{90}) were extracted from the longevity dataset through parametric survival regression models (*Survival* package for *R*; (Therneau, 2013). The period of activity along the year was analysed with Generalized Linear Models (Negative Binomial distribution), with months as the explanatory variable (the number of captures expressed as the proportion of adults caught per month cancelled the year effect), and followed by post-hoc comparisons to discriminate monthly differences. As there were no significant differences between the two sampling locations ($\chi^2 = 0.023$, $P = 0.88$), this factor was dropped.

Variation in the number of carried eggs along the year was tested through Generalized Linear Models (Poisson distribution), with sampling dates and body size index as explanatory variables. The body size index corresponds to individuals' coordinates on the first axis (accounting for 50.5% of total variance) of a Principal Component Analysis computed on the five morphological measurements (*FactoMineR* package for *R*;(Lê et al., 2008). The ratio between females without eggs and gravid females

for each sampling date was analysed through a Generalized Additive Model (*mgcv* package; (Wood, 2004).

Finally, Linear Models were undertaken for each of the three categories of body stores (proteins, glycogen and triglycerides), with month and year as explanatory variables.

All statistics were computed with *R* 3.0.1 (R Development Core Team, 2008).

3 - Results

a) *Estimation of adult longevity*

The mean adult longevity under controlled conditions at 8 °C was 241 ± 6 days (about 8 months; LT_{50}), with 10% of insects living 413 ± 9 days (LT_{90}) (Fig. 2). The last individual died after 710 days, almost two years after it was sampled from the field. The sex of each individual could not be determined a posteriori, because the fortnight check caused substantial decaying and scavenging of dead individuals, that did not allowed reliable sex determination.

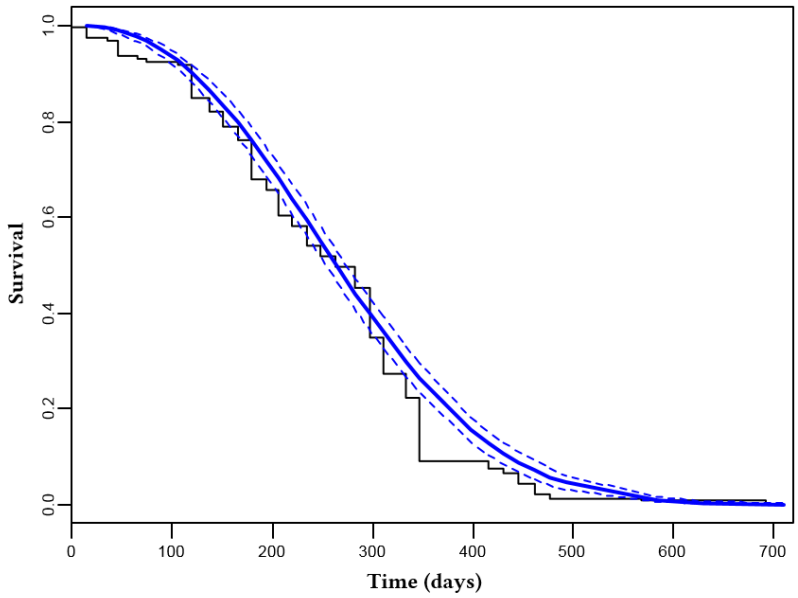

Fig. 3 Survival data of 500 adult *Merizodus soledadinus* reared in controlled conditions (8°C, 12:12 photoperiod, regularly supplied with food and water) from March 2013 until the death of the last individual (solid line), and predicted probability of survival (solid bold curve; dashed curves : 95% CI)

b) *Annual activity in natural environments*

From 2005 to 2015, a total of 7927 ground beetles were caught, with noticeable but non-significant inter-annual variability (from 367 specimens in 2009 to 1223 in 2014, $\chi^2 = 1.39$, $P = 0.24$). A seasonal pattern of activity was observed (Fig. 3, $\chi^2 = 114.95$, $P < 0.001$), with activity of adult *M. soledadinus* caught during Austral summer (mean total catches per month from October to March (± SD), N = 12.74 ± 9.93 individuals) being about three times higher compared to Austral winter (mean total catches per month from April to September (± SD), N = 4.74 ± 5.03 individuals). The highest density was observed in December (N = 20.27 ± 13.69 individuals) and January (N = 15.44 ± 7.86 individuals) every year.

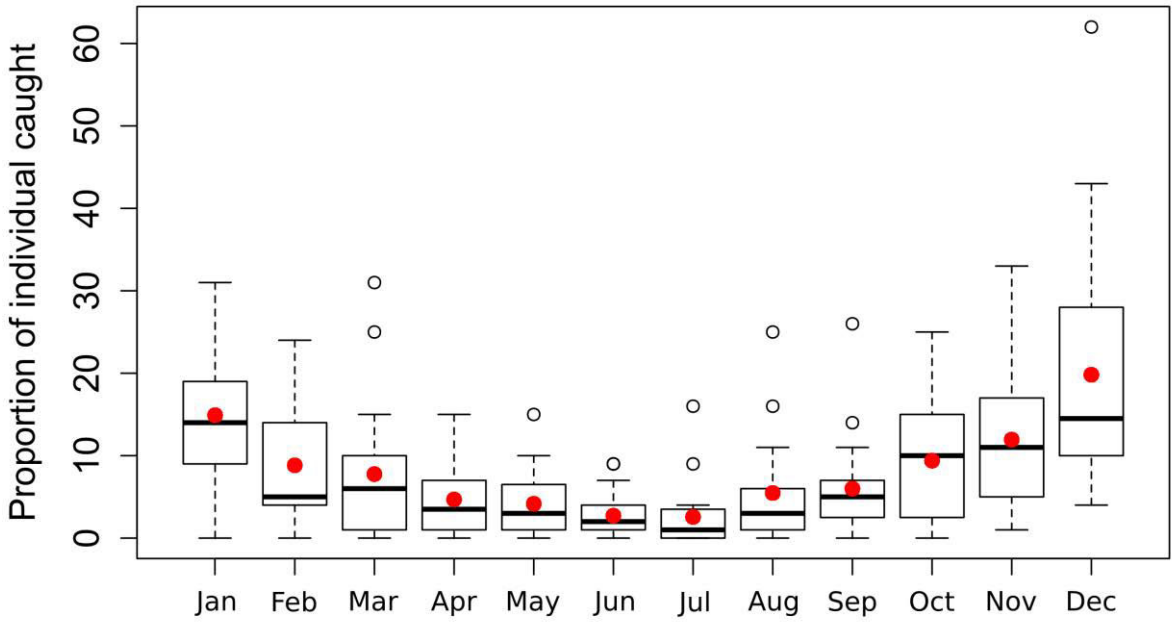

Fig. 4 Annual activity of adult *Merizodus soledadinus* measured as the proportion of individuals trapped each month on total catch of each year, over a 10-years period Boxes represent median, upper and lower quartile of the monthly distribution. Dots correspond to the predicted values of the statistical model (GLM, negative binomial distribution)

c) *Reproductive status of the females along the year*

Gravid females of *M. soledadinus* carried a mean of 8.94 eggs (SD = 3.56, N = 367), among which 6.73 (SD = 3.22) were mature, *i.e.* located in the distal part of the abdomen. Neither the total number of eggs per female (z = 0.507, $P = 0.612$; see Online Resource Fig. ESM_2) nor the number of larger eggs (z = 0.127, $P = 0.899$) varied by month. Larger females (with a higher body size index) carried significantly more eggs (z = 5.406, $P < 0.01$). The ratio of females without eggs was marginally non-significant over the year (Generalized Additive Model with sampling date as the smoothed explanatory variable, $F_{8,02} = 2.2$, $P = 0.067$).

d) *Annual variation of body stores*

Amounts of proteins were remarkably stable over the year (months: $F_1 = 0.009$, $P = 0.927$, year: $F_1 = 0.333$, $P = 0.565$) (Fig. 4). Glycogen contents did not vary by months ($F_1 = 0.544$, $P = 0.85$) nor by years ($F_1 = 1.295$, $P = 0.26$). Finally, triglyceride contents progressively increased at the onset of summer (from August to October), then decreased over the summer (from November to March), with significant differences between months ($F_1 = 11.08$, $P < 0.01$). This pattern tended to be consistent over the three studied years ($F_1 = 2.74$, $P = 0.1$).

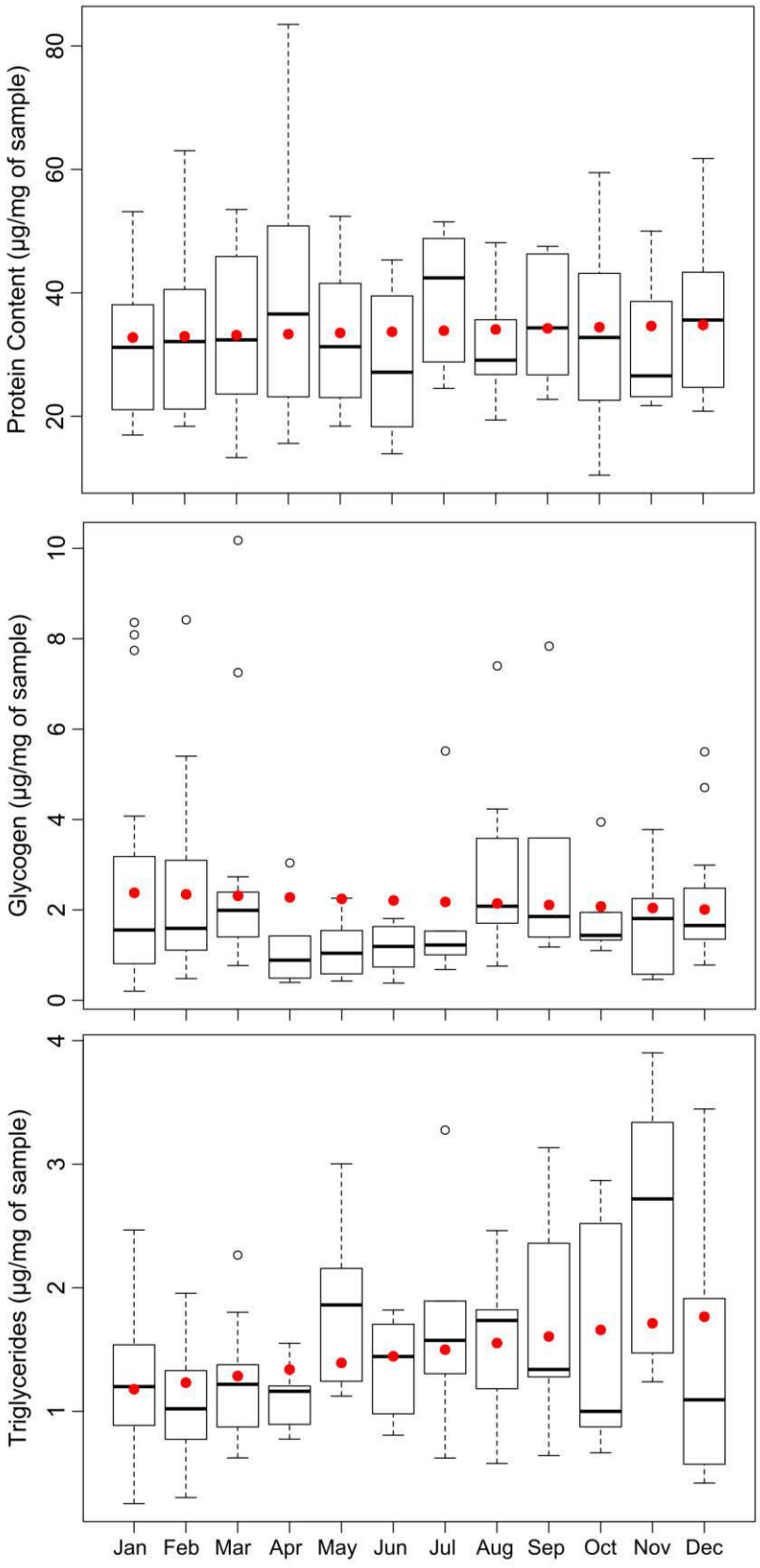

Fig. 5 Monthly variations in three reserve compounds contents (proteins, glycogen and triglycerides) in adult *Merizodus soledadinus* sampled from December 2010 to March 2012. Colorimetric assays were performed on the same samples. Boxes represent median, upper and lower quartile of the monthly distribution. Dots correspond to the predicted values of the statistical model (GLM, Negative Binomial distribution)

4 - Discussion

Our study found a median adult longevity of about eight months. Adults were regularly fed and supplied with water over the duration of the experiment, and benefited from favourable thermal conditions. These experimental conditions may have elevated longevity as compared with the likely life span under natural conditions. On the other hand, at the Kerguelen Islands, *M. soledadinus* can feed on abundant prey in most localities and only suffers from occasional mice predation in some invaded microhabitats (Le Roux et al., 2002) and cannibalism. Moreover, adults are also able to endure natural thermal conditions cooler than those applied in the present experimental setting (Lalouette et al., 2012; Laparie & Renault, 2016), which may even slow down ageing processes and augment longevity.

Rearing trials of *M. soledadinus* were not successful, preventing us to estimate the exact life span and total life cycle of this species. In the present work, the sampling period maximised the number of teneral individuals caught to allow more realistic estimations, and the calculated adult life span is more likely underestimated than overestimated. No peak of mortality was observed during the very first months of the experiment, and mortality largely increased one year after insect collection (between days 310 and 340, ca. 33% of beetles died). Our measurement is consistent with life span estimations earlier reported by Ernsting (1993) based on egg load and assumptions on the life cycle of *M. soledadinus* in South Georgia. Data reporting carabid longevity are scarce, and range from one to seven years depending on the species, with estimations based on mandibular or claw wear, reproductive status and number of overwintering periods (Thiele, 1977; Lövei & McCambridge, 2002). The adult life span of *M. soledadinus* is similar to native carabid beetles, including *Amblystogenium pacificum* and *A. minimum* which belong to the same feeding guild in other sub-Antarctic islands (Davies, 1987). The invasive *M. soledadinus* seems, however, to display a lower seasonality in its life cycle as well as an increased investment in reproduction (number of eggs in the ovaries) compared with this carabid beetle native from Crozet archipelago.

Our field observations at the Kerguelen Islands (Laparie, Lebouvier, Renault, unpublished data) correspond to Ernsting's (1993) reports from South Georgia, and likely suggest an emergence period ranging from September to April, with the main peak of tenerals around February-March. Gravid females were found across the whole year, and the number of eggs in the ovaries did not vary significantly over time. This finding suggests that egg-laying can theoretically occur at any time and result in high phenological variance, although higher summer activity may exacerbate reproduction. This result is in contrast with the semelparity of the ground beetle hypothesised by Ernsting (1993), i.e. one reproductive season per individual. Females of long-lived carabid species are usually capable of multiple cycles of egg maturation. For instance, *Abax parallelus* (Löser, 1970), *Pterostichus chalcites* (Kirk, 1975)or *A. pacificum* (Davies, 1987) exhibit several consecutive egg-laying periods. Associated with a low periodicity of the life cycle, multiple egg-layings may conceal any inter-individual variance

in seasonality of the egg load. In our work, the number of eggs per female was higher than reported in South Georgia by Ernsting (1993), even though we examined fewer individuals. Climatic conditions are harsher in South Georgia than at the Kerguelen Islands (Chown et al., 1998), and may thus be more restrictive and impair investment in reproduction.

The year round trapping of adult *M. soledadinus* is consistent with previous studies on this species (Kerguelen Islands: Chevrier et al., 1997; South Georgia: Ernsting, 1993), and demonstrated continuous activity of adults. From our 10-year investigation, the peak of trapping occurred during summer, and is likely associated with a peak in the reproductive season of this insect (as demonstrated through similar methodology in *Pterostichus madinus* in Luff [1973]). Importantly, Jeannel (1940) sampled all life stages of *M. soledadinus* at once in summer at the Kerguelen Islands, also suggesting the long-lasting developmental period of this species and the aforementioned phenological variance within populations. Lower pitfall catch rates during winter periods together with an average adult life span over one year suggest seasonal changes in adults' locomotor activity related to thermal conditions rather than a demographic decrease. This assumption is consistent with the results of Ottesen (1990) who found a positive correlation between daily activity of this insect and ground temperature.

The decreased activity of *M. soledadinus* during the Austral winter, whether it is caused by, or consequential to, the colder environmental conditions, may contribute sparing body reserves in a period where several native and introduced prey species are inactive or scarcer. Interestingly, amounts of glycogen and protein did not exhibit large variations over the year, whereas starving *M. soledadinus* were earlier characterised by declining amounts of sugars and lipids (Laparie et al., 2012). Our present data suggest that adults continuously feed during the supposedly less favourable winter season. *Merizodus soledadinus* have been described as opportunistic predators fond of Diptera maggots (Chevrier et al., 1997), implying the possibility of diet adjustments towards other available prey in winter periods. For instance, they have been shown to feed on the native beetle *Hydromedion sparsutum* on South Georgia (Convey et al., 2011). This idea is supported by variations of triglycerides whose amounts did not vary much over the seasons. The only remarkable variation was observed at the onset of the summer period, with amounts of these stored lipids being augmented and more variable, likely revealing the return to maximal activity in parallel with increased food availability. Meanwhile, our samples were composed of whole body extracts and were not controlled for sex ratio. It is thus hard to determine the relative lipid part reflecting egg maturation, and the relative contribution of each gender to these lipid variations. Finally, resorption of unlaid eggs in females (Rosenheim et al., 2000), or cannibalism on laid eggs by males and females, cannot be excluded during extended periods of food deprivation.

This study brought additional insights into the life histories of *M. soledadinus* at the Kerguelen archipelago. Imagos of this generalist predator are long-lived. Gravid females are found during the

whole year, and our data do not allow to pinpoint restricted egg-laying phenologies or clear age of reproduction. The life cycle appears to be marked by low seasonality, which concurs with the low climate seasonality in the sub-Antarctic Kerguelen Islands. At present, the recruitment and mortality rates of new adults remain unknown (egg, larval and pupal mortality rates have not been assessed, nor adult mortality in natural conditions). However, knowing that adult abundances are substantial in most sites colonised by *M. soledadinus* (up to >200 individuals caught per person per 10 minutes of active search; Mathieu Laparie, Marc Lebouvier, David Renault, personal observation), and that larvae are also voracious predators, we can only glimpse the threat this carabid beetle poses on arthropods communities at the Kerguelen Islands (Lebouvier et al., 2011). Consistently, this species represents a major threat to native communities at South Georgia, by threatening native populations of the beetle *Hydromedion sparsutum* (Convey et al., 2011). The transfer of this voracious predator to other islands must be avoided through extensive biosecurity measures (Laparie & Renault, 2016).

Acknowledgments

The French Polar Institute Paul-Emile Victor that supports our program IPEV 136 'Subanteco' is warmly thanked. This study was granted by InEE-CNRS (Enviromics call, 'ALIENS'; Zone Atelier Antarctique et Subantarctique). Part of the experiments were conducted at the ABGC platform (UMR CNRS EcoBio). Our samplers and breeders of *Merizodus soledadinus* at Port-aux-Français from 2013 to 2015 - Elsa Day, Lauriane Laville, Solène Lebreton and Camille Thomas - are warmly thanked.

Supplementary material

Tab. ESM_1 Summary of the hand-collected samples used to investigate the reproductive status of females during the year. The number of individual captured is pooled for each month if the number of samples per month exceeds one. The sex ratio of each month is calculated as the proportion of females per sample

Sampling date	No. of samples per month	No. captures	Sex ratio	No. dissected females	No. females with only mature eggs	No. teneral females caught	No. eggs in teneral females caught
Dec-10	2	214	0.20	25	5		
Jan-11	1	124	0.32	20	5		
Feb-11	1	63	0.46	20	5		
Apr-11	3	173	0.28	60	17	5	0;5;12;0;0
May-11	2	94	0.25	31	3	1	0
Jun-11	1	18	0.36	10	1		
Jul-11	1	41	0.37	20	2		
Aug-11	2	81	0.38	37	7		
Sep-11	2	107	0.25	36	1	2	0;0
Oct-11	2	99	0.27	35	11		
Nov-11	1	54	0.21	14	2		
Dec-11	2	109	0.29	40	15		
Jan-12	2	36	0.50	36	13	3	0;0;0
Feb-12	2	86	0.29	35	7	4	0;0;0;0
Mar-12	2	86	0.28	34	4	2	16;12
Apr-12	2	91	0.17	10	0		
May-12	1	63	0.14	9	3		

Fig. ESM_1 Difference in melanisation between an adult (left) and a teneral individual (right)

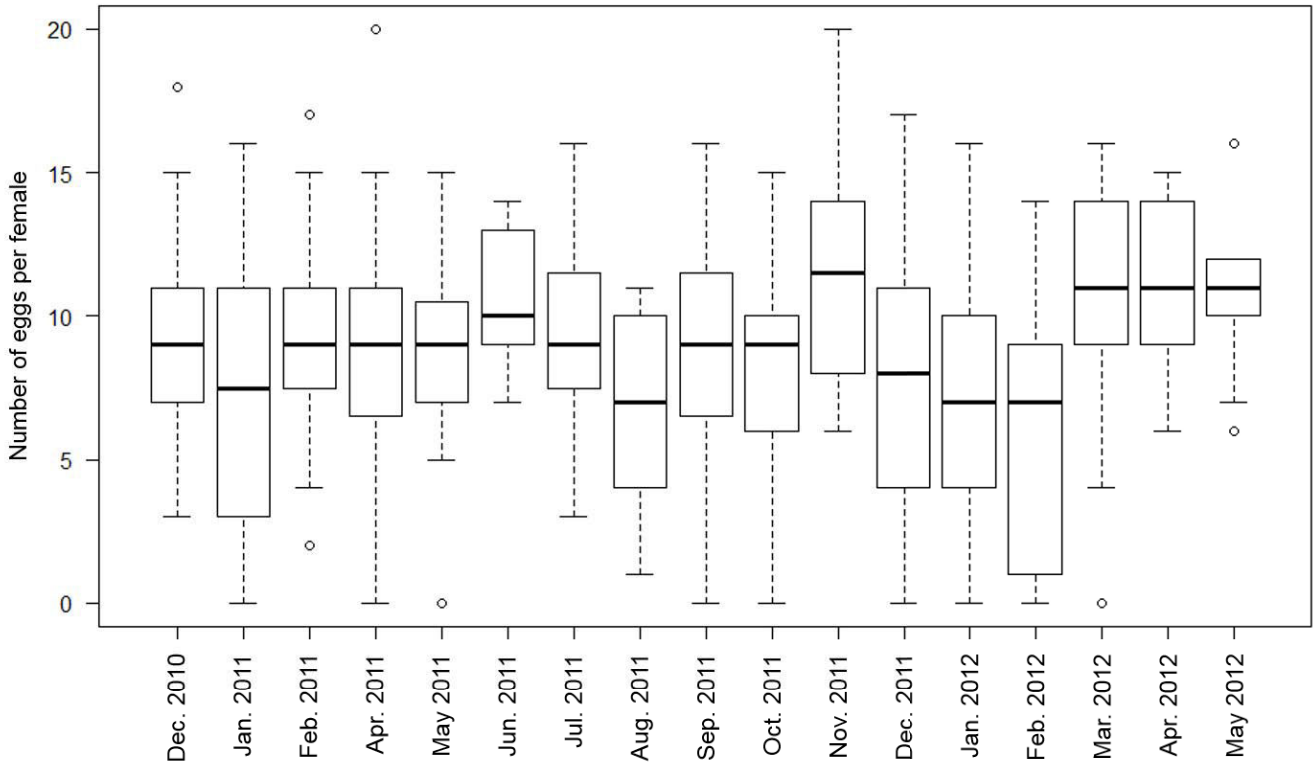

Fig. ESM_2 Mean egg load in dissected female *Merizodus soledadinus* sampled monthly between December 2010 and March 2012 (20 individuals per sampling date, when possible)

Chapter 2

Biogeographic reconstruction and populations genetics of the invasive carabid beetle *Merizodus soledadinus*

T. Ouisse, D. Renault, F. Hendrickx

This chapter presents the advancement status of the investigations on population genetics of *M. soledadinus*.

Highlights

- Comparison of genetic diversity patterns between specimens of *M. soledadinus* from (i) Patagonia, South Georgia and Kerguelen and (ii) ten populations distributed along the invasion gradient at the Kerguelen Islands.
- Individuals from the Kerguelen Islands are less genetically diverse than in South Georgia and in Patagonia; investigated populations along the invasion gradient are not genetically structured.
- Supplementary locations need to be added to (i) complete the biogeography of this species and (ii) further test for human-assisted introductions

1 - Introduction

Investigations on population genetics recently became a recurrent feature in invasive species studies. These studies allow to infer the geographic origin of the sampled specimens, and routes of introduction of alien species (Estoup & Guillemaud, 2010). *In fine*, these data allow us gaining *a posteriori* precious information on the history of invasion cases (Dlugosch & Parker, 2008; Hufbauer & Sforza, 2008; Lombaert et al., 2011; Cristescu, 2015). Genetic studies are also cornerstones for understanding the level of selection alien species endured, and their subsequent evolutionary ecology. Altogether, it allows a better understanding of the invasion and adaptation processes, and on the effects of evolutionary forces on species genomes (Lee, 2002; Sax et al., 2007).

Invasive species are predominantly non-model species, *i.e.* species for which none or few genetic information is available. Early investigations on population genetics of invasive species were principally based on RFLP, AFLP and microsatellites (see for example Evans et al., 2004). Those techniques offered the opportunity to get access to the genetic information of non-model species, and allowed to delineate fundamental genetic processes occurring in alien populations and invaded communities (Sakai et al., 2001). However, these techniques have been progressively replaced. The production of species-specific markers is time-consuming, and the quick technological advances in sequencing (Next-Generation Sequencing, NGS) have now provided rapid access to much larger sets of genetic markers. Next generation sequencing thus confers more power to present genetic analyses (Davey et al., 2011; Rašić et al., 2014; Rius et al., 2015).

NGS methods flood ecological studies since roughly a decade, because of the large spectrum of application they offer, in terms of biological models (everything that contains DNA can be sequenced), and in terms of fields of topics it covers (phylogeny, evolution, biodiversity assessments, biological invasions). Reduced-representation techniques allow to sequence a subsample of a genome to estimate neutral genetic diversity (Single Nucleotide Polymorphism markers, SNP) across a large number of populations and individuals (Davey et al., 2011). Among these techniques, Restriction-Associated DNA sequencing allows to sequence flanking regions (150 to 300bp) of restriction sites spread across the genome. These sites are theoretically at the same place for all individuals of a given species. RAD sequencing should thus target the same regions across all individuals (except in cases of mutation in the restriction site). By aligning sequenced fragments (called RAD tags) across individuals, it is possible to isolate polymorphic loci, thus genotyping and estimating genetic diversity from markers spread across the genome (Baird et al., 2008; Etter et al., 2011).

These methods are particularly appropriate for the non-model species *M. soledadinus*. The native range of this insect is assumed to extend from Patagonia to the Falkland Islands. Accidental introductions occurred during the XXth century at the Kerguelen Islands and in South Georgia, where *M. soledadinus* was first observed in 1939 and 1963 respectively (Jeannel, 1940; Darlington, 1970). According to historical records, *M. soledadinus* was probably uniquely introduced in 1913 at a single

location of the Kerguelen Islands, during the transfer of sheep from the Falkland Islands (Jeannel 1940). South Georgia being much closer to the Falkland Islands and Patagonia than the Kerguelen Islands, it is possible that several introduction events have occurred in this island. Delineating the genetic relationships between native and introduced populations of *M. soledadinus* should permit to confirm these historical-based suppositions. As introduced specimens are constituted by a subsample of the original population (successful propagules that survived the transfer phase), they often display reduced genetic diversities compared with the specimens from the population of origin (Wares et al., 2005; Dlugosch & Parker, 2008). In the present study, we hypothesise that *M. soledadinus* from Kerguelen Islands and South Georgia host lower genetic diversities than their counterparts sampled from their native areas (Patagonia, Falkland Islands). This pattern should be more pronounced for the individuals of the Kerguelen Islands which population is supposed to be the result of a single introduction event, conversely to South Georgia where several introductions from different locations of the native range of *M. soledadinus* may have occurred. To address this hypothesis, we compared the genetic diversity in samples of this species collected in Patagonia, Kerguelen Islands (Port Couvreux, the introduction site at the Kerguelen Islands, and South Georgia.

At the Kerguelen Islands, *M. soledadinus* is present on coastal areas of the eastern part of this archipelago, and is colonising a variety of habitats inland and in altitude. Yet, when specimens were first spotted at the scale of the Kerguelen Islands in 1939, they were only present at the supposed introduction site of Port Couvreux. Regular biodiversity assessments on the Kerguelen territory allowed to reconstruct the chronosequence of the range expansion of this ground beetle, and to estimate colonisation periods of distinct locations. Laparie et al. (2010, 2013) described a morphological gradient of increased body size across different sites of regressive residence time (also see Chapter 2 of this PhD thesis). Such a phenotypic variation associated to the invasion front wave is putatively attributed to spatial sorting. Despite the short time elapsed since the introduction of *M. soledadinus* at the Kerguelen Islands, and the probably low genetic diversity of the founder population, we hypothesise that the expansion dynamics produced population differentiation along the invasion gradient. Specifically, we expect a progressive decrease of genetic diversity from Port Couvreux (core population) up to the edge populations that are present in the most recently colonised localities. This hypothesis is investigated in this study by comparing the neutral genetic diversity in sampled collected in ten locations characterised by different colonisation periods, and by testing if isolation by distance occurred.

2 - Material and methods

a) Sampling locations

A total of eight RAD-seq libraries were created, each containing 16 individuals. The RAD-seq protocol was first tested on this non model species, with 16 individuals sampled between December 2010 and January 2011 from different locations of the Kerguelen Islands (results not presented here).

After acknowledging the success of the procedure, two other libraries were designed to answer the biogeographic question, and each library contained six individuals from Patagonia (four from Ushuaïa, one from Punta Arenas and one from Rio Rubens), five individuals from South Georgia and five individuals from Kerguelen Islands (Port Couvreux). Specimens from Patagonia were collected in January 2009 from three locations, as reported in Fig. 1. The samples from Port Couvreux (Kerguelen Islands) consisted of DNA extracts from 2008. Specimens from South Georgia were sampled in 2009 in the vicinities of the King Edward research station (Grytviken). Five RAD-seq libraries were designed to investigate population genetics within populations of the Kerguelen Islands, and included individuals sampled between November and December 2014 in nine localities (eight individuals per locality) characterised by different estimated colonisation period by *M. soledadinus* (Fig. 1). Residence times correspond to the first observation of *M. soledadinus* individuals at the different locations, and ranges between 102 to 3 years since the introduction date of *M. soledadinus* at the Kerguelen Islands. A supplementary site was added, Val Studer North, located halfway between the formerly colonised site of Port Elizabeth and the most recently colonised site of Val Studer. Genetic proximity shared by those individuals with specimens from Port Elizabeth and Val Studer would inform on the colonisation path along the Val Studer valley.

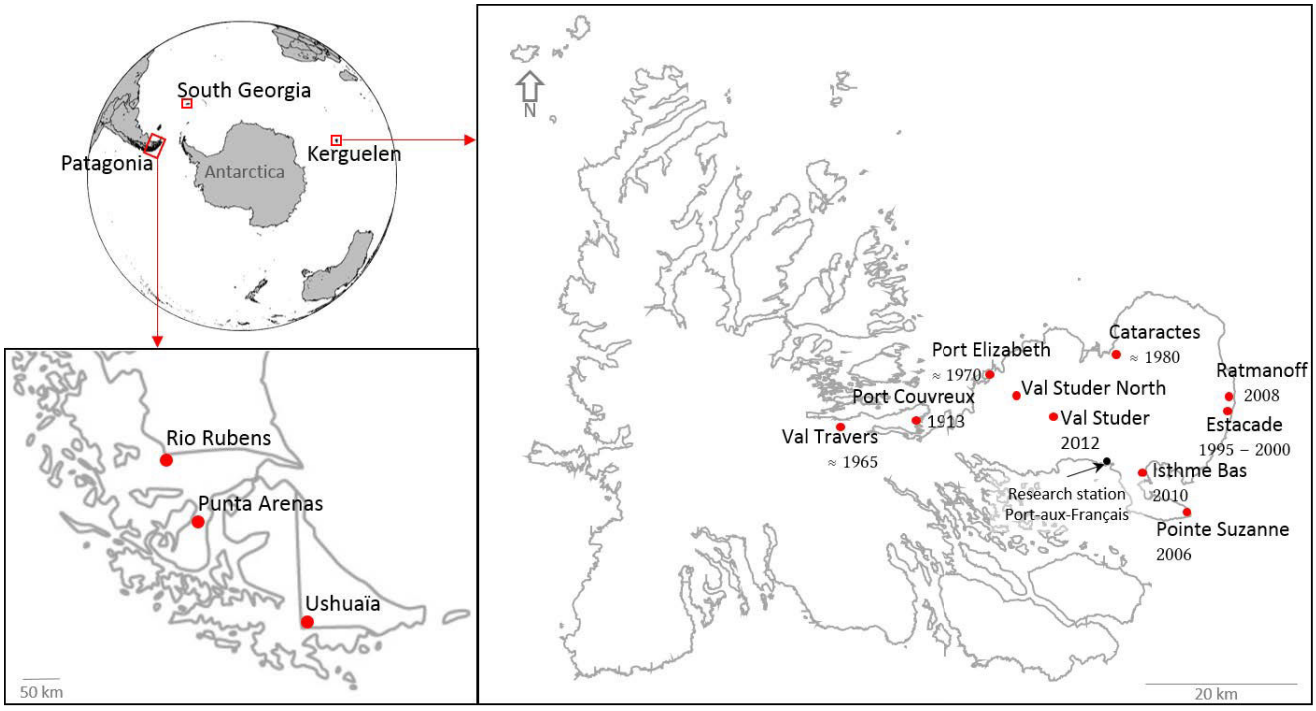

Fig. 1 Localisation of the sampling sites in Patagonia and at the Kerguelen Islands. The years indicated represent the estimated colonisation periods of the different sites of the Kerguelen Islands (except for Val Studer North, which remains unknown)

b) RAD-sequencing libraries preparation

RAD libraries were prepared from the protocol of Fuentes-Utrilla (unpublished), based on Etter et al. (2011) and Baird et al. (2008). Fundamental methodological steps are outlined below. After DNA extraction (for whole body samples) and purification (Quiagen DNeasy Blood & Tissue kits), we

checked DNA concentration (Qubit fluorometer) and quality (electrophoresis) in each sample. Extracted DNA was digested by the restriction enzyme SbfI (which targets restriction sites 5' CCTGCAGG 3'). Individuals barcodes were then ligated to each side of the restriction fragments, which were then randomly sheared (sonication). Through electrophoresis, fragments 300 < length < 700bp were selected and purified. On the 3' and 5' ends of each DNA strand, an adenine was ligated, on which secondary barcodes (specific of the library and containing flowcell annealing sites) ligated. Finally, 14 to 16 cycles of a test PCR allows to verify the quality of each library created.

The first RAD library was 300bp paired-end sequenced on an Ilumina MiSeq platform (The Gene Pool, Ashworth lab, University of Edinburgh) and the seven other libraries were 100bp paired-end sequenced on an Illumina HiSeq1500 platform ("Biogeography" libraries (N=2) were sequenced The Gene Pool, Ashworth lab, University of Edinburgh; "Kerguelen populations genetic" libraries (N=5) were sequenced at the Medical Genomics Institute, UZ Antwerp Hospital, Belgium).

c) Sequenced data processing

To process the RADtags, we first assembled the 2x300bp MiSeq reads of a single individual with the highest coverage (Bel01) to generate a set of reference tags. Reads were assembled using Velvet (Zerbino & Birney, 2008).

Used that way, this software allows to reconstruct the approximate 700bp fragments sequenced on each side of restriction sites, creating two contigs per restriction site. Velvet parametrization required to define the Kmer length (the length of each subunit of a contig, to assemble together), the insert length (the length of the sequenced fragments). We defined the following parameters: Kmer length = 31bp, insert length = 450bp, coverage cutoff = 10 reads per position minimum. We verified the quality of the assembly by plotting the coverage and length of the obtained contigs and retained only those with a read length > 450bp and a coverage between 10 and 110. After filtering we retained 2040 high-quality contigs.

Next, we mapped the reads of each individual against the reference RADtags with BWA (Burrows-Wheeler Aligner software, Li & Durbin, 2010). SNPs were subsequently called with GATK (Genome Analysis ToolKit software, McKenna et al., 2010; DePristo et al., 2011; Van der Auwera et al., 2013), which resulted in a VCF file that was used for appropriate filtering to retain a set of high-quality SNPs. One individual from South Georgia (SGF_5) was removed from the dataset, as very few reads mapped against the reference tags. This suggests that the sequenced specimens belongs to a different species (*Trechisibus antarcticus*) that is morphologically closely related to *M. soledadinus*.

From this step, data analyses were separated, with one part focusing on the biogeography dataset on one hand (individuals sequenced in the second and third libraries), for which wo obtained 1077 SNPs shared by 90% of the individuals, and on the other hand the individuals sampled in the ten distinct localities of the Kerguelen Islands, for which we obtained 4271 SNPs shared by all sequenced individuals.

Summary statistics were computed using Geneop 4.4 (Raymond & Rousset, 1995; Rousset, 2008), including Hardy-Weinberg exact tests, inbreeding coefficients (F_{IS}) and differentiation between populations (pairwise F_{ST}). The genetic structure of populations was investigated independently for the two main datasets (Biogeographical scales and Kerguelen scale) using Structure 2.3.4 (running settings: admixture ancestry model and correlated allele frequencies; Pritchard et al., 2000) and StructureHarvester (Earl, 2012) was used to determine K, the number of clusters. Heterozygosity data were extracted from the VCF file using VCFTools. The sampling location (for the Biogeographical analysis) and the effect of residence time of populations (for the Kerguelen population's genetic analysis) on heterozygosity values were statistically tested with linear regressions (R. 3.3.0, R Core Team, 2016). Isolation by distance was investigated on the populations from Kerguelen Islands by testing the correlation between the genetic distance (F_{ST}) matrix and the matrix of Euclidian geographic distances (mantel tests, 999 permutations, package *vegan*), using R 3.3.0.

3 - Results

a) Biogeography

A total of 561 SNPs were shared by at least 90% of the individuals sampled in the three studied biogeographic regions (Kerguelen Islands, South Georgia, Patagonia). These three biogeographic regions respected Hardy-Weinberg requirements, and are genetically distinct, as shown in the Structure output (optimum K=3 populations, see Fig. 2). When the number of clusters is set to K=2, Structure groups together specimens from the two introduced geographic areas (South Georgia and Kerguelen Islands), which differ from the native area (Patagonia). Individual assignments to population clusters shows the greater homogeneity of Port Couvreux individuals compared with individuals assigned to South Georgian and Patagonian locations.

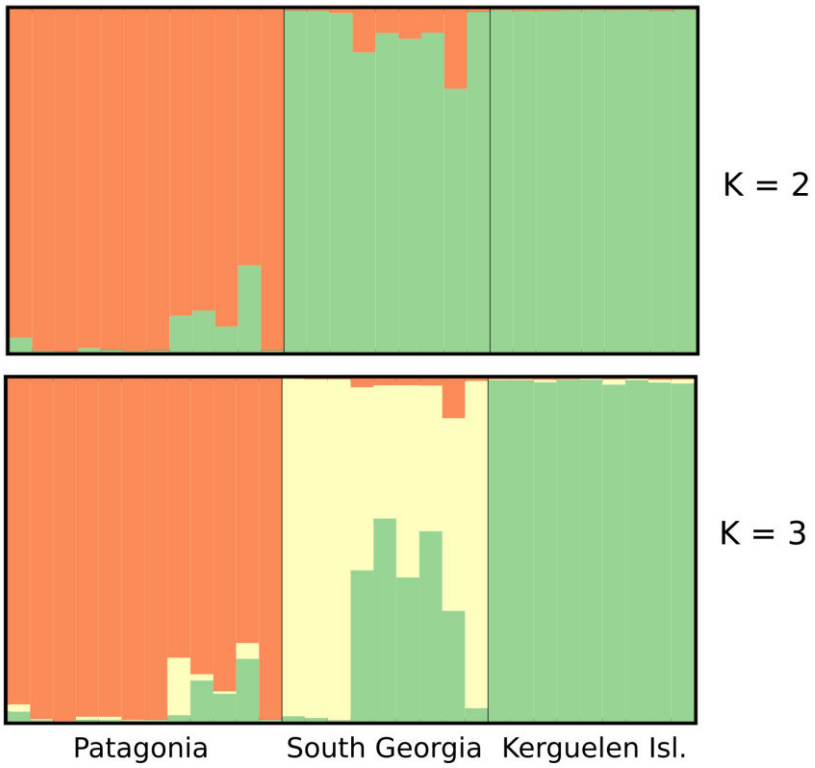

Fig. 2 Structure outputs for RAD-seq data from adults of *M. soledadinus* sampled in Patagonia (native geographic area), South Georgia and Kerguelen Islands (introduced areas, with first estimated introduction in 1963 and 1913, respectively). The proportion of assignation (vertical axis) of each individual (vertical bars) is presented for K=2 and K=3 population clusters

Genetic differentiation of the specimens from the three biogeographic area is also outlined by pairwise F_{ST} values between the three populations. A stronger differentiation was observed between Patagonia and South Georgia (F_{ST} =0.1), or between Patagonia and Kerguelen Islands (F_{ST} =0.12) than between South Georgia and Kerguelen Islands (F_{ST} =0.0016).

The inbreeding coefficient F_{IS} is negative for all individuals from the three geographic regions (Kerguelen Islands =-0.029, Patagonia = -0.106, South Georgia = -0.133). These results show increased heterozygosity compared with random mating for the three populations, especially South Georgia.

Heterozygosity values revealed significant differences among insects of the three localities (χ^2=7.35, P <0.01). *Post-hoc* tests outline that individuals from Kerguelen Islands host lower levels of heterozygosity than individuals from Patagonia (t =-3.78, P<0.01), the two other comparisons being non-significant (South Georgia-Patagonia: t =-2.23, P =0.08; Kerguelen Islands-South Georgia: t =-1.54, P =0.28). However, we found that heterozygosity of individuals is correlated with the mean sequencing depth (see Fig. 3), probably skewing the results.

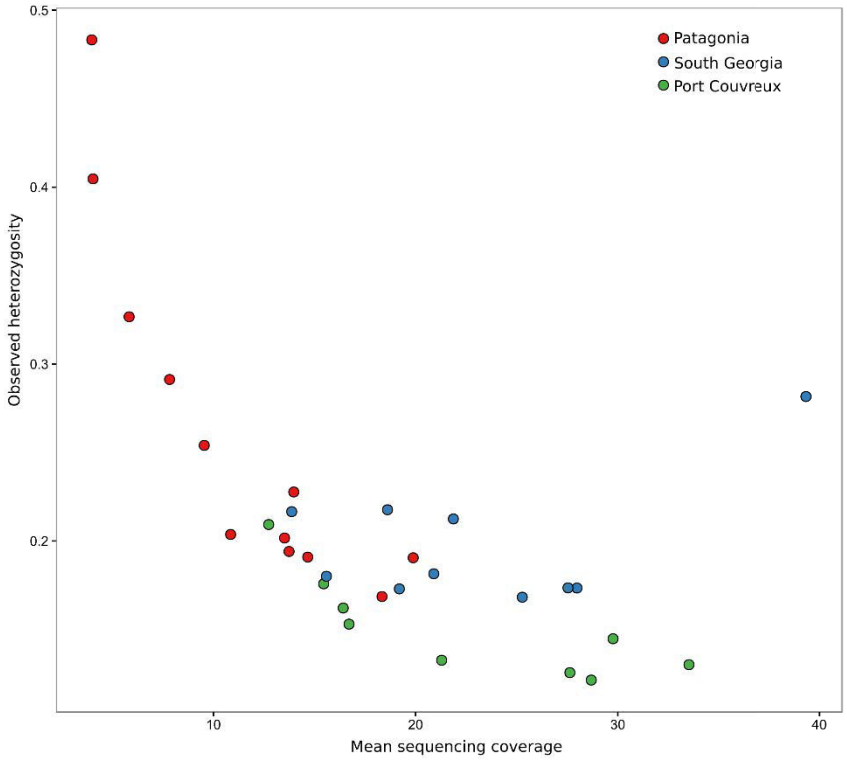

Fig. 3 Correlation between observed heterozygosity and mean sequencing depth in the 31 individuals sequenced for the biogeographical reconstruction of specimens of *M. soledadinus*. The five individuals presenting a mean coverage of 10 were discarded from further analyses of heterozygosity values

When considering only individuals with a mean sequencing coverage superior to 10, individuals from Kerguelen Islands show patterns of heterozygosity significantly different from individuals from the two other locations (Kerguelen Islands-South Georgia: t =-3.52, P <0.01; Kerguelen Islands -Patagonia: t =-3.14, P<0.05; see Fig. 4).

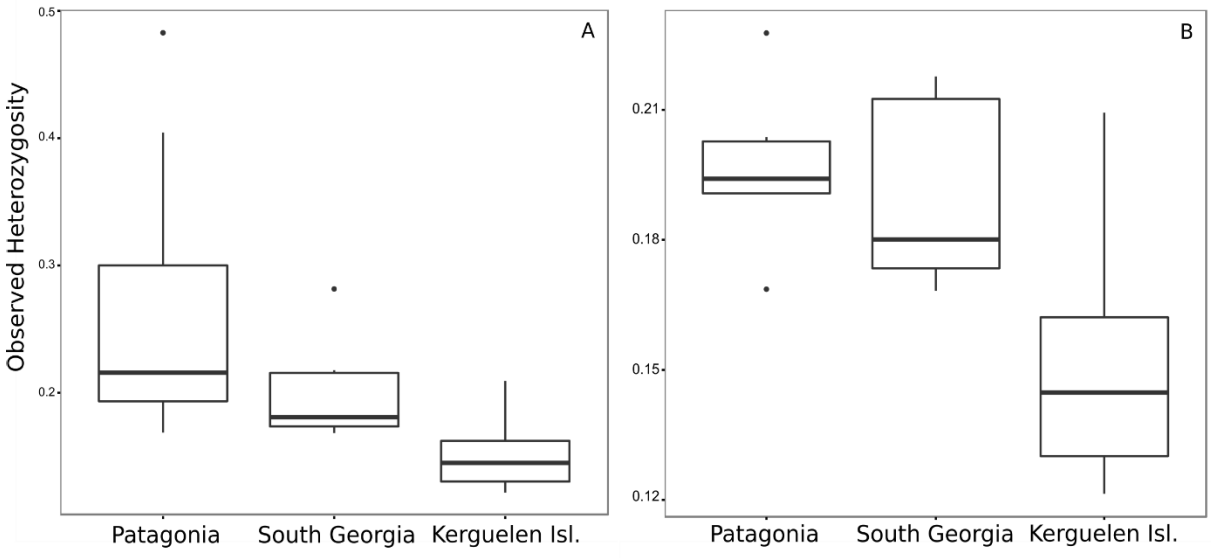

Fig. 4 Heterozygosity values of adult *M. soledadinus* sampled at the Kerguelen Islands, South Georgia and Patagonia. (A) Thirty-one individuals were sequenced and were all included into the analysis and (B) only the individuals with a mean sequencing depth above 10 (five Patagonian individuals were discarded) were kept for running the analysis

b) Population genetics of M. soledadinus *at the Kerguelen Islands*

A total of 1825 polymorphic loci were shared by all individuals (10 populations × 8 individuals). The Structure analyses do not highlight particular population structure (optimum K=1 population). Yet, the eight individuals from Estacade show more similarities among them than with the individuals from the nine other populations. Individuals from two recently established populations, Ratmanoff and Val Studer, also show assignation patterns that slightly differ from individuals of the other populations (see Fig. 5).

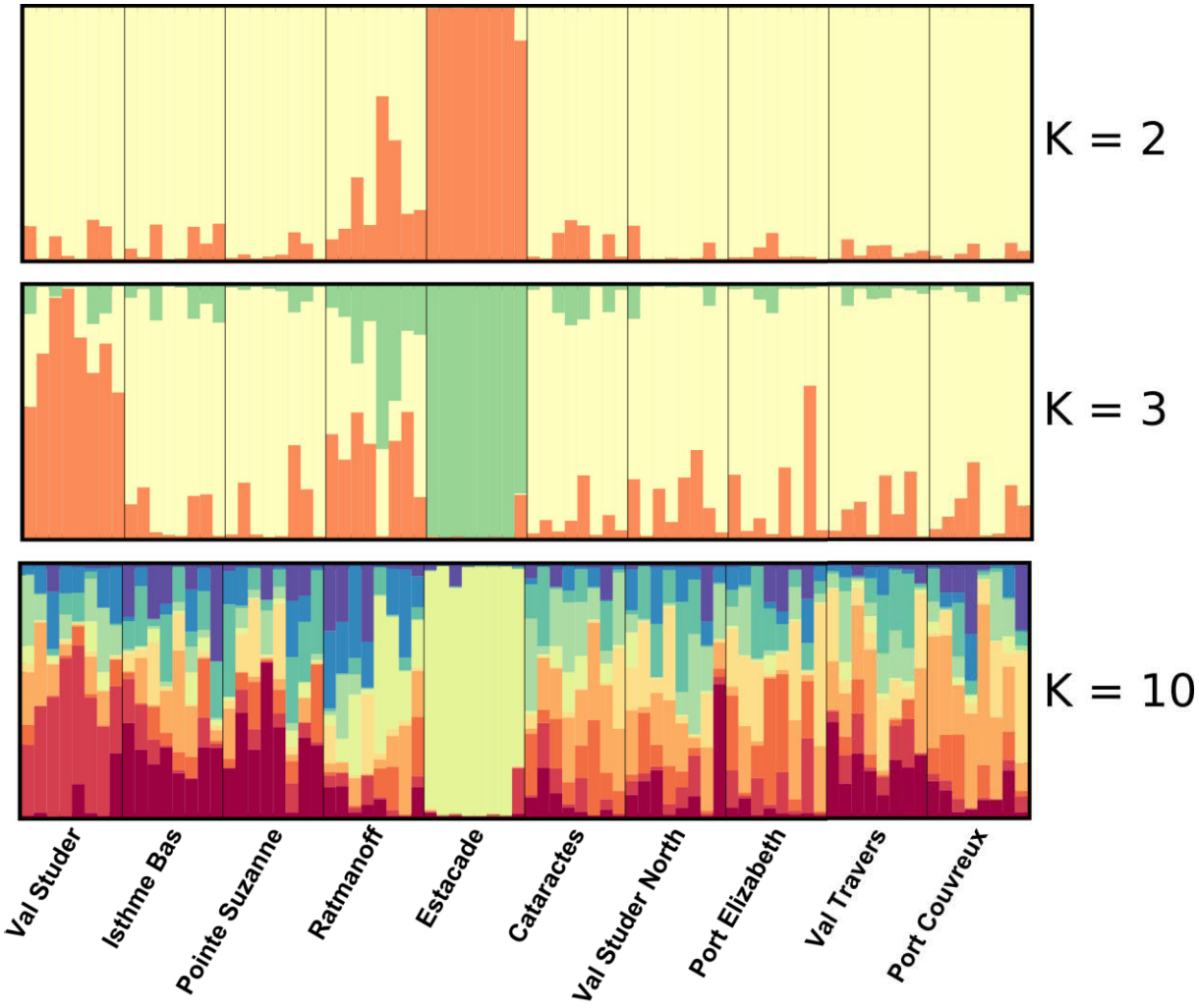

Fig. 5 Structure output of the sequenced individuals sampled from ten populations at the Kerguelen Islands (eight individuals per population), for K=2, K=3 and K=10 clusters

All populations respect Hardy-Weinberg conditions, with trend towards a deficit in heterozygotes in the individuals from the most recently colonised location of Val Studer (P = 0.057). This population is also the only one presenting a positive F_{IS} (0.004). The lowest F_{IS} is displayed by Estacade (-0.065). Overall, the 10 populations from Kerguelen have a mean F_{IS} =-0.027 (see Fig. 6).

The residence time of the ten populations is significantly correlated with mean individual heterozygosity values (F=18.82, P<0.001). Higher values are found in individuals from Port Couvreux, and heterozygosity values decrease along the invasion gradient (see Fig. 7).

No correlation between pairwise F_{ST} and geographic distance matrix was found (Fig. 8). Isolation by distance involving all populations (r = 0.17, *P* = 0.15) revealed the isolation of the individuals from the population of Estacade. The test performed on all populations except Estacade was also inconclusive (r = -0.026, *P* = 0.49).

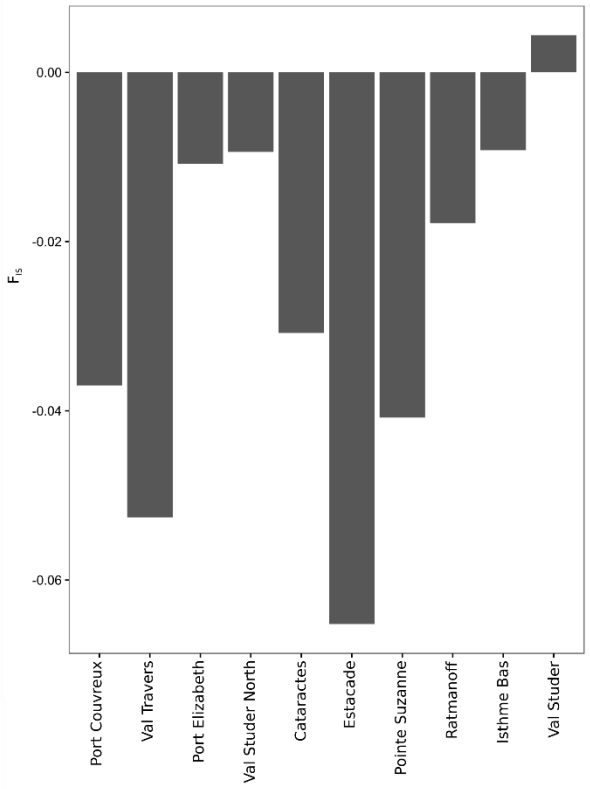

Fig. 6 Inbreeding coefficient values (F_{IS}) of the sequenced adult *M. soledadinus* sampled from ten localities at the Kerguelen Islands

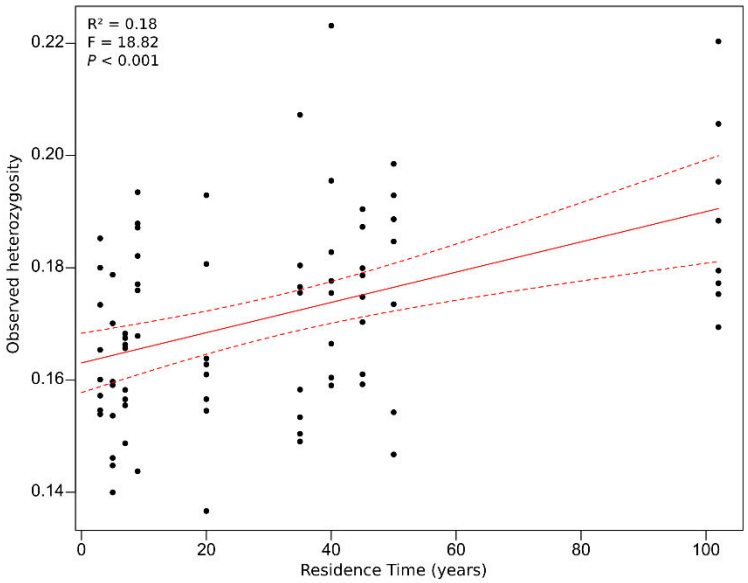

Fig. 7 Correlation between observed heterozygosity and estimated residence time of *M. soledadinus* adults sampled from ten localities at the Kerguelen Islands. Mean individual heterozygosity values are represented (black dots), as well as the regression curve (red line) for heterozygosity values and residence time, together with 95%CI (dashed lines)

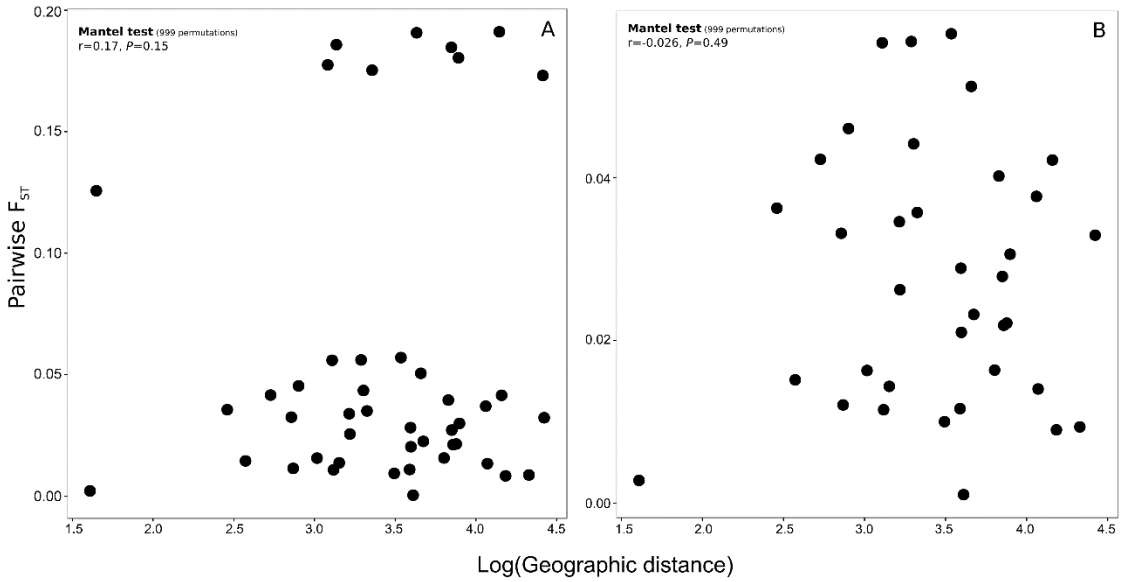

Fig. 8 Isolation by distance along the invasion gradient of *M. soledadinus* at the Kerguelen Islands. (A) All populations are considered and (B) population of Estacade is excluded from the analysis. Statistical results of Mantel tests are given for the two tests

Concerning the colonisation directionality in the Val Studer valley, pairwise F_{ST} among the localities of Port Elizabeth, Val Studer North and Val Studer reveal increased genetic proximity between Port Elizabeth and Val Studer North (F_{ST} = 0.0022). Conversely, the genetic proximity was lower between Val Studer North and Val Studer (F_{ST}=0.036).

4 - Discussion

a) Biogeography

The comparison of genetic diversity and population structure among three geographic regions, out of the four occupied by *M. soledadinus,* revealed that the Kerguelen Islands host a population less diverse than in Patagonia and South Georgia. Specimens from South Georgia show a higher level of genetic diversity compared with their counterparts from the Kerguelen Islands. This is interesting, as *M. soledadinus* was first observed 53 years ago in South Georgia, whereas it has been observed 103 years ago at the Kerguelen Islands. The higher genetic diversity of insects from South Georgia could be explained by multiple introduction events to this island, located closer to the Falkland Islands, and thus more likely to have experienced more transportations and introductions of *M. soledadinus*. It is also important to mention that Falkland Islands represent an important route of transit in order to get access to the subantarctic South Georgia. If multiple introduction events have occurred at South Georgia, this would have increase the chances of admixture among insects of distinct genetic lineages, thus explaining the increased levels of diversity observed in this island. This hypothesis will have to be confirmed by incorporating into our analyses specimens of *M. soledadinus* from the Falkland Islands.

Coverage values highlighted that sequences of South Georgian and Patagonian individuals were generally of lower quality than the sequences from specimens from the Kerguelen Islands, and would probably require resequencing. This lowered quality could partly explain the lower amount of polymorphic loci detected in these individuals compared with the analysis of population genetics along the invasion gradient at the Kerguelen archipelago. This pattern could also be due to the use of an individual from Kerguelen as “reference genome”; this procedure could have increase the degree of divergence among populations, further lowering the proportion of RAD-tags matching.

b) Population genetics of the invasive carabid beetle M. soledadinus *at the Kerguelen Islands*

As hypothesized, adult *M. soledadinus* from Port Couvreux are characterized by the highest level of genetic diversity, as compared with the other sequenced specimens, from ten localities of the Kerguelen Islands. Again, this finding supports the hypothesis of a single introduction event in the locality of Port Couvreux, further followed by natural stepping-stone dispersal events. There is no detectable population structure among specimens from the ten sampled populations, which could be explained by a combined effect of reduced genetic diversity and recent history of this introduction. In parallel, the absence of genetic structure at the scale of the Kerguelen Islands could also depict a high

frequency of migration events among populations, a process that could be reinforced in some localities by human transportations. These two processes would greatly contribute to homogenising the pool of genetic diversity.

Conflicting this absence of genetic structure, heterozygosity values display a strong pattern of reduced genetic diversity along the invasion gradient. This result supports the hypothesis of range expansion from the initial and unique introduction locality of Port Couvreux, with progressive colonisation of uncharted sites by few dispersers. However, no patterns of isolation by distance were detected. Euclidian geographic distances may have constituted imperfect metrics for this species whose range expansion patterns primarily follow coastal habitats. In future investigations, least-resistance path analyses, attributing higher resistance values to altitudinal habitats for example, could be more appropriate to understand the colonisation process of *M. soledadinus* at the Kerguelen Islands (Wang et al., 2009; Spear et al., 2010). Finally, spatial sorting resulting from the ongoing range expansion of this species may mask population differentiation if it is based on neutral genetic markers only. Indeed, spatial sorting acts as a selection mechanism, and few genes might be involved in phenotypic sorting (Shine et al., 2011). A first exploratory step into this direction would be to search within the dataset for outlier loci, as realised in (Vandepitte et al., 2014).

We suggest that adding genetic sequences of individuals from the research station of Port-aux-Français would be valuable and informative. Knowing the genetic diversity of specimens from this locality would allow to estimate the genetic composition of *M. soledadinus*, these individuals being an important dissemination source for the colonisation of several localities of the Kerguelen Islands, more particularly for islands of the Golfe du Morbihan. Such implementation is indispensable for assessments of human-mediated introductions from the research stations.

The specimens from Estacade have a very low genetic diversity (but from the F_{IS} values, this is the least inbred population of the ten sequenced populations, which is a conflicting result). This population was first observed between 1995 and 2000, and most probably results from the transportation of some few individuals (maybe a single gravid female) by humans to this locality. Indeed, this locality hosts a hut where research activity took place, and is, in addition, on the main path that as used bytractors for resupplying 4-8 times per year the site of Ratmanoff (the largest studied colony of king penguin at the Kerguelen Islands). The activities at the locality of Estacades have been stopped rapidly, all of them being now concentrated at Ratmanoff. Multiple introduction events from Port-aux-Français could explain greater genetic diversity at Ratmanoff, where individuals were first observed around 2008.

Individuals sampled at Val Studer show reduced genetic diversity, and a positive inbreeding coefficient. We hypothesised that this recently established population could either result from anthropogenic introduction from the research station, or from natural dispersal from the north coast (Port Elizabeth). To tackle this assumption, individuals from the intermediary site of Val Studer North

were included into our analyses, to evaluate the possible arrival of *M. soledadinus* from the north (Port-Elizabeth). Our data confirmed this range expansion path, revealing inland colonisation from the north coast, and also suggest that the inland population of Val Studer was probably introduced by humans

To conclude, *M. soledadinus* invaded most of the eastern part of the Kerguelen archipelago in roughly 60 years. The geographical expansion is now growing faster, possibly as a result of spatial sorting, human transportations, increased beetle's demography and climate change. These results seemingly cast a dark shadow on the native arthropod communities of the western, still uncolonized, part of the archipelago, which could soon glimpse the moving tips of *Merizodus*' antennas. To complete this study, we will have to incorporate additional data so that the biogeography of this species will be completely covered, and thus even better understand population dynamics of *M. soledadinus* at the Kerguelen Islands.

Acknowledgments

Carl Vangestel and Katrien de Wolf are warmly thanked for their help and advises during data processing and analyse. Mathieu L., Christophe D. and Bernard K. are also thanked, for their help during fieldwork. This study benefited from a InEE-CNRS grant (Enviromics 'ALIENS'), and was supported by the Synthesys program, by the French Polar Institute (IPEV, program 136) and by CNRS (Zone Atelier Antarctique et Subantarctique)

Chapter 3

Large-scale phenotyping along the invasion gradient of the carabid beetle *Merizodus soledadinus* at the Kerguelen Islands

T. Ouisse, F. Hendrickx, D. Renault

In prep.

Highlights

- Characterisation of several morphological, behavioural and physiological traits in populations sampled along the invasion gradient
- Individuals sampled in localities recently colonised have different morphology, locomotor activity, starvation resistance and metabolite amounts than individuals from formerly established populations
- Results from this new integrative approach show distinct phenotypic differentiation depending on the residence time of populations, possibly explained by spatial sorting

1 - Introduction

Biological invasions have become a central research theme in ecology and evolution over the past decades (Handley et al., 2011; Chown et al., 2015). The number of exotic species invading terrestrial and aquatic habitats has increased strongly, and even if some exotic organisms may have beneficial effects on invaded communities and ecosystems (Simberloff & von Holle, 1999; MacDougall & Turkington, 2005), most of them have been reported to cause serious threats to native biota (see Vilà et al., 2011). Beyond the pure ecological causes and consequences of biological invasions, this phenomenon often represents valuable situations for real-time monitoring of phenotypic and genotypic changes associated to establishment and range expansion of invasive organisms (Sax et al., 2007; Davis, 2009). In this context, species adaptations and spatial sorting of invasive populations along colonisation gradients are fascinating, yet also intriguing, and these processes have become a hot topic in invasion studies (Phillips et al., 2006; Monty & Mahy, 2010; White et al., 2013; Tingley et al., 2014).

Dispersal performance represents a prominent aspect that triggers the distribution of range-shifting species, and likely coevolves with functional traits driving fecundity and/or growth rates (Hanski et al., 2006). General concepts of population ecology suggest that leptokurtic dispersal distributions represent a common pattern (Kot et al., 1996), with large amounts of individuals residing in a given locality (*e.g.* the 'residents'), and decreasing amounts of specimens that effectively disperse (*e.g.* the 'dispersers'). However, our current understanding of the evolutionary processes generating such pheno- or genotypic variation in dispersal ability remains incomplete, especially under non-equilibrium conditions like range expansion and invasion (Kinlan & Gaines, 2003; Fitzpatrick et al., 2012). Pioneer studies were conducted on the cane toad, and reported that dispersal performance was increased with an exponential fashion in Australia. Specifically, cane toads sampled at the invasion front are characterised by higher body sizes, locomotor performance, and more straight dispersal paths than their non-dispersing counterparts (Phillips et al., 2006; Alford et al., 2009; Brown et al., 2014). The correlation between dispersal ability and body size extends to serval insect species (Juliano, 1983; Gutiérrez & Menéndez, 1997; Zera & Denno, 1997). In addition, in some insect taxa, the expression and allelic diversity of the gene encoding phosphoglucoisomerase (PGI) has been proven a powerful estimator of dispersal capacity of individuals (Mitikka & Hanski, 2010; Niitepõld, 2010).

In most factual cases of invasion phenomena, human-assisted dispersal events can impede our understanding of alien species range expansion (Walther et al., 2009), which has thus often been hung over through modelling (Travis & Dytham, 2002; Burton et al., 2010; Perkins et al., 2012). While theories suggest that spatial sorting may contribute by selecting dispersive phenotypes at the front distribution margins, the magnitude of this process is expected to be impeded by a range of life history trade-offs that may either facilitate or constrain the species' invasion success, with subsequent fitness consequences (*i.e.* constrained energetic outputs; Burton et al., 2010). Subantarctic islands are ideal laboratory-system to address this kind of questions. Briefly, these islands are remote from continental

ecosystems and main commercial routes. Human presence is recent, and when there are research stations, buildings are restricted to a narrow part of these islands.

Despite reduced anthropogenic activity and presence at the subantarctic islands, a number of invasive plants, mammals and insects has been introduced (Frenot et al., 2005), and their subsequent natural range expansion monitored (Convey et al., 2011; Lebouvier et al., 2011). At the Kerguelen Islands for instance, the carabid beetle *Merizodus soledadinus* was introduced in 1913 at a single locality, yielding a century of dispersal, without human assistance in a significant number of colonised localities. In this insect species, Laparie et al. (2013) investigated morphological differences between individuals whose populations differed in time since establishment (hereafter referred to as residence time). These authors found that adult *M. soledadinus* sampled from recently established populations were bigger than individuals sampled from formerly established populations. Even if several hypotheses can be mounted for explaining these body size differences, this consistent pattern among different animal taxa of distinct trophic regimes requires further attention.

Spatial sorting is the prominent explanation for explaining changes in body sizes during range expansion. Individuals of bigger sizes often have better abilities to disperse (higher dispersal performance and success) over larger geographic ranges. When established, the assortative mating of these individuals further contribute to maintain higher body sizes, as demonstrated experimentally in the damselfly *Coenagrion scitulum* (Therry et al., 2015). Generation after generation, front populations thus carry biological traits associated with enhanced dispersal performance and capabilities. For instance, empiric studies reported longer legs, bigger thoracic muscles, increased muscular efficiency, stronger tolerance to starvation or to other abiotic stressors, and higher body stores (Hill et al., 1999; Haag et al., 2005; Phillips et al., 2006; Hughes et al., 2007; Kelehear et al., 2012; Lombaert et al., 2014). Conversely, in formerly established populations, the higher population density and the qualitative and quantitative decline of food resources increase intra specific competition. This can supposedly lean towards the production of higher amount of eggs by females (to counteract completion-induced mortality of the juveniles) and faster developmental rates of the juveniles, both processes acting jointly and resulting in adults of smaller body sizes (Amundsen et al., 2012).

In a recent work, Van Petegem et al. (2016) ran metabolomics approaches to explore the metabolic phenotypes of the two-spotted spider mite along an invasion gradient. While mites were reared in common garden, these authors reported evident signs of metabolic adjustments, with specimens from the invasion front tending to exhibit metabolic activity oriented towards more intense energetic production. Meanwhile, populations were not replicated, which, together with the unknown residence time of the sampled specimens, hampers the drawing of general conclusions.

In the present study, we aimed at conducting a large-scale phenotyping of specimens of *M. soledadinus* sampled from populations with different estimated residence times. As a prerequisite, we

first wanted to confirm the results obtained by Laparie et al. (2013), using different populations. In a second step, we explored the potential effects of residence time on several different phenotypic traits related to, or that should promote dispersal performance, *i.e.* locomotor performance, propensity to disperse, starvation resistance, enzymatic assays, and GC-MS-TOF metabolic phenotyping. To our knowledge, this is the first study that integrates all of these elements together. We believe that this is a useful way to detect variations among functional traits in invasive populations of alien insects, and delineate the complex eco-evolutionary trade-offs responsible for sex-specific dispersal-related phenotypes. We hypothesise that morphological proxies and dispersal performance correlates (locomotor activity, dispersal propensity) will be higher in specimens of *M. soledadinus* from the invasion front. Then, as dispersal performance is supposedly increased along the invasion gradient, we should see changes in some of the following aspects: increased amounts of metabolic stores, modulation of enzymatic flux capacities, dispersal-associated shift in the trophic pathways to promote reserve accumulation and dispersal decisions.

2 - Material and Methods

a) Sampling locations

Adult *M. soledadinus* were hand-sampled under rocks on the main island of the Kerguelen archipelago (see Fig. 1). Insects were collected from a total of ten localities along the invasion gradient, with a residence time ranging from 103 to 4 years. Insect sampling occurred during the austral summers 2014-2015 and 2015-2016. Due to the constrained field logistics in subpolar regions, slight changes in the visited localities occurred between these two working periods, meaning that not all populations were systematically tested for all the large-scale phenotyping. Meanwhile, this does not affect our experimental design, which focused on the residence time of the specimens rather than the localities *per se*.

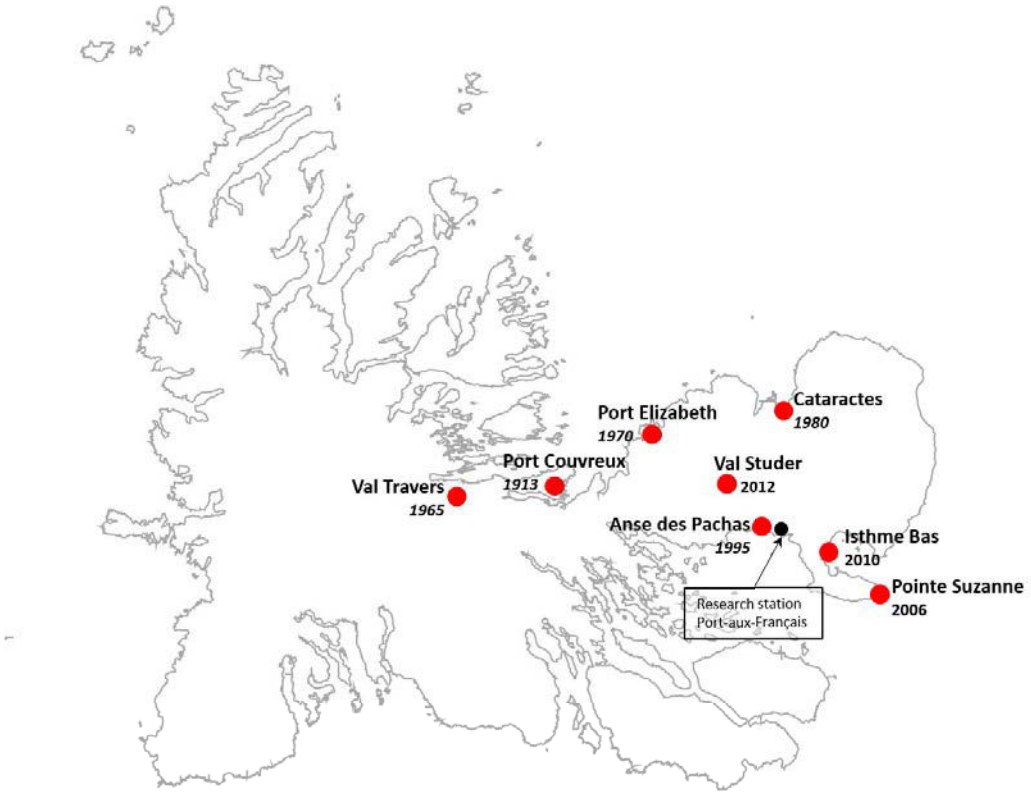

Fig. 1 Localisation of the sampling sites on the main island of the Kerguelen archipelago, and estimated (years in italic) or verified date of colonisation by *M. soledadinus*

In most of the localities (see Fig. 2), individuals were sampled and directly plunged into ethanol 96° (N = 15 microtubes per locality, each containing pools of five individuals) for being further used for morphometric measurements and metabolic phenotyping. These samples were then stored at -20 °C and sent to mainland France. In parallel, approximately 300 individuals per locality were caught and brought back alive to the research station of Port-aux-Français. The specimens were then installed into rearing boxes (17 × 23 × 9.5 cm) on humid sand; adults were fed *ad libitum* with dipteran maggots or enchytraeids. All populations were reared under the same controlled conditions in a walk-in chamber at 8 ±2°C, photoperiod 12:12 (L:D). These individuals were then used for assessment of starvation resistance, locomotor activity and dispersal propensity.

Fig. 2 Recap chart of populations implemented in each phenotypic trait investigation. The estimated residence time of *M. soledadinus* at each locality is given in brackets (years)

Sampling Sites	Morphometry	Starvation Resistance	Locom. Activity	Contr. Dispersal	Enzymatic Activity	Metab. Fingerprinting
Port Couvreux (103)	X	X	X	X		X
Val Travers (51)		X				X
Port Elizabeth (46)	X		X		X	
Cataractes (36)	X	X	X	X	X	X
Anse des Pachas (21)			X	X		
Pointe Suzanne (10)	X	X	X	X		X
Isthme Bas (6)	X	X	X	X	X	X
Val Studer (4)	X	X		X	X	X

Enzymatic assays required alive ground beetles, and equipment that was not unavailable at the research station of Port-aux-Français. Thus, ground beetles from five populations (N = 600 individuals from Port Elizabeth, Cataractes, Pointe Suzanne, Isthme Bas and Val Studer) were sent to the mainland France at 4 °C in April 2016, and further installed in controlled chambers in the dark at 6 °C and fed with *Calliphoridae* larvae.

b) Morphometric measurements

This experiment was conducted to confirm the morphometric gradient described in Laparie et al. (2010, 2013), where specimens were characterised by increased body sizes from core to edge ranges. The same analytical procedure as the one described in Laparie et al. (2013) was applied on imagos sampled at Port Couvreux, Port Elizabeth, Cataractes, Pointe Suzanne, Istme Bas and Val Studer. From the 75 *M. soledadinus* adults conditioned into ethanol 96°, 15 males and 15 females were isolated and measured under stereo microscope (Stemi 2000- c, obj 10×23 ZEISS, Germany) coupled to a camera (AxioCam ERc 5s, obj 0.5x, pixel size 2.2µm × 2.2µm, ZEISS, Germany). Morphological traits were measured from vectorial layouts with Zen software (ZEISS, Germany). The following traits were measured: total length (from the basis of the labrum to the end of the right elytra), interocular distance, thorax width and length, length of the right elytra, width of the abdomen and length of the right femur from the first and third pair of legs (Fig. 3).

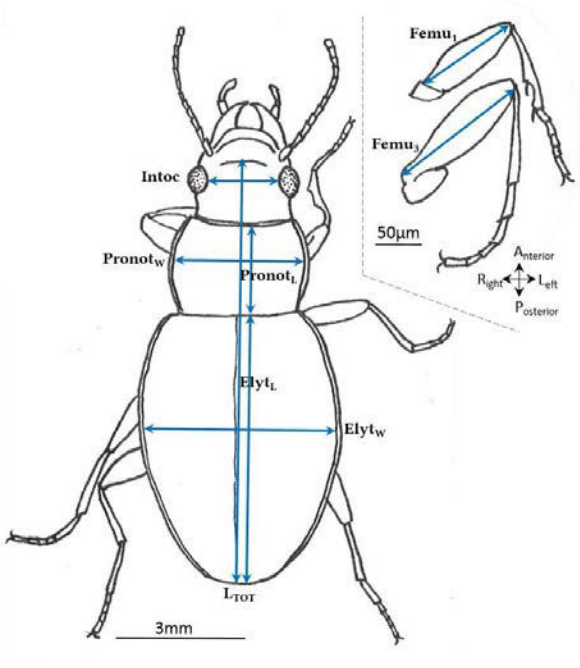

Fig.3 Graphical representation of the measured morphometric traits on adults of *M. soledadinus*. On the dorsal view, the following traits were measured: total length (L_{TOT}), interocular distance (Intoc), width and length of the pronotum ($Pronot_W$ and $Pronot_L$, respectively), the length of the right elytra ($Elyt_L$) and the width of the abdomen ($Elyt_W$). After removal of the right foreleg and hind leg, both metafemur were measured, under a larger magnification ($Femu_1$ and $Femu_3$, respectively)

c) *Starvation resistance*

We compared starvation resistance of adult *M. soledadinus* sampled from six populations along the invasion gradient. We focused on specimens from three populations from formerly colonized habitats (Port Couvreux, Cataractes and Val Travers), and on specimens from three recently established populations (Pointe Suzanne, Isthme Bas and Val Studer). After being brought back to the research station of Port-aux-Français, sampled specimens were kept under controlled conditions (see **1-a**) for 10 days to standardize the physiological condition of the individuals (trophic status, thermal acclimation, humidity and salinity conditions, density of the insects, etc…). Then, 50 *M. soledadinus* from each population were weighted (Sartorius

Analytic, Goettingen, Germany, d=0.1 mg) and randomly installed individually into petri dishes bottomed with a paper disc, with a source of water (a microtube of water stoppered with a cotton ball). Every five days, dead individuals were counted. The dynamic of weight loss during starvation was investigated through weighting the 15 lighter and the 15 heavier individuals of each population every five days.

d) Comparison of the locomotor activities among populations

A Trikinetiks (LAM 10, TriKinetics Inc., Waltham, MA) device measuring via light beams the number of crossings of individuals in glass tubes was used to individually compare locomotor activities of 30 individuals per population. Specimens from the localities of Port Couvreux, Port Elisabeth, Cataractes (formerly colonised habitats) and Anse des Pachas, Pointe Suzanne and Isthme Bas (edge ranges) were considered for this experiment. Individuals were randomly and individually installed in a glass tube (⌀ 10mm, length 10cm) sealed by a black plastic cap at one end, and by a humid cotton ball at the other end. Four individuals of each population were tested for 24 hours into this experimental device, resulting in seven replicates ran consecutively. The initial dataset reports the number of movements recorded at a resolution of 30 seconds. At the end of the experiment, individuals were weighted, killed by cold shock at -80°C and sexed.

e) Dispersal propensity in controlled conditions

The propensity to disperse was investigated in individuals from Port Couvreux, Cataractes (formerly colonised habitats), Anse des Pachas, Pointe Suzanne, Isthme Bas and Val Studer (recently colonised habitats) during the austral summer 2016. The experiment took place in the walk-in chamber whose conditions have been described above. Ten individuals of each population were randomly chosen and installed in 'home boxes': opaque boxes (⌀ 6cm, height 4cm) with a sandy bottom and a rock as a shelter. One aperture led to a 1meter-long tube (⌀ 16mm), ending with a downward bend, in such a way that individuals at the end of the tube fall into a glass bottle and cannot turn around. The number of individuals fallen in the glass bottle were counted twice during the two days this experiment lasted, and a final counting included the individuals in the tube and the individuals that remained in the 'home' box. Six replicates of ten individuals per population were conducted, three between the 13/01/2016 and the 15/01/2016, for which the number of dispersers was checked after 20, 29.5 and 44 hours in the experimental device, and three others between the 04/03/2016 and the 06/03/2016, with observations made after 12, 24 and 36 hours in the experimental device. Only nine out of the ten individuals initially installed in the experimental design for one replicate of Pointe Suzanne and Cataractes were numbered at the end of the experiment. In one replicate of Isthme Bas, only four individuals were found at the end of the experiment, and this replicate was discarded.

f) Enzymatic assays

A total of six enzymatic assays were performed on fresh individuals brought back from the Kerguelen Islands to the Metropolitan France: total antioxidant activity, hexokinase (HK) and phosphoglucose

isomerase (PGI) activities were measured on whole body extracts of *M. soledadinus*. Citrate synthase (CS), malate dehydrogenase (MDH) and fumarase activities were measured on mitochondrial extracts from adult *M. soledadinus*. These assays were restricted to specimens from four populations, *i.e.* Port Elizabeth and Cataractes (formerly colonised habitats) on the one hand, and Isthme Bas and Val Studer (recently colonised habitats) on the other hand. Specimens from Pointe Suzanne were also added in the total antioxidant and HK assays. Three sets of 24 ground beetles (12 males and 12 females) were isolated by sex and last fed at least four days prior to extractions: one set for the antioxidant assay, one set for both HK and PGI, and finally one set for the mitochondrial extraction and the following enzyme quantifications. For each set, 12 samples constituted by two alive beetles of the same sex were weighted and maintained on ice just before extraction.

- Total antioxidant activity, phosphoglucoisomerase and hexokinase

Quantifications of total antioxidant activity, PGI and HK were performed following manufacturer's instructions (Cayman assay kit number 709001, and Sigma assay kits MAK103 and MAK091, respectively). Whole body samples were extracted in 300µL of extraction buffer supplied with the kits, using a bead-beating device (Retsch™ MM301, Retsch GbmH, Haan, Germany) and homogenised for 1.5min at 25Hz. Samples were then centrifuged (10 min, 1 500*g*, 4 °C).

For the total antioxidant activity, ten microliters of the supernatant were used for the assay, and activity was quantified from the drawn trolox standard curve (absorbance red at 750nm).

For the PGI assay, three µL of the supernatant were diluted into 47µL of assay buffer, before being used for the quantification of PGI activity. A NADH standard curve was done to estimate the rate of conversion of fructose-6-phosphate to glucose-6-phosphate, with the absorbance red at 450nm, during a 9min reaction (except for two replicates of Isthme Bas, two replicates of Cataractes and one replicate from Val Studer, for which the reaction rate was calculated over a 6 min interval).

For the HK assay kit, 6µL of extraction supernatant, diluted in 44µL of assay buffer were used to quantify Hexokinase activity, using a NADH standard curve to estimate the rate of Glucose to Glucose-6-Phosphate conversion, with the absorbance red at 450nm, during a 9min reaction (except for a 6min reaction for a replicate from Cataractes).

- Mitochondrial extractions and quantification of citrate synthase, fumarase and malate dehydrogenase activities

Mitochondrial extractions were performed following the instructions of the Cayman MitoCkeck Mitochondrial isolation kit n°701010. Samples constituted by two alive beetles were grinded into 1.5mL of ice-cold homogenization buffer using a bounce homogenizer. Subsequent homogenate was filtered through a 300µm mesh and transferred into a polycarbonate centrifuge tube before being centrifuge for 10min at 10 000*g* at 4 °C. Supernatant was then discarded and the pellet was re-dissolved in 300µL of isolation buffer. Mitochondrial enzymatic assays were performed following the instructions of the Sigma assay kits MAK193 (Citrate Synthase), MAK206 (Fumarase) and MAK196 (Malate

DeHydrogenase - MDH). We used 50µL of mitochondrial extract to quantify citrate synthase and fumarase activities, but for the MDH, 15µL of mitochondrial extract were diluted in 35µL of isolation buffer before the assays. The citrate synthase activity was quantified at 412nm (25 °C), whereas MDH and fumarase activities were quantified at 450nm (37 °C). For the three assays, the absorbance was red every minute for 10 minutes. Only the linear part on the kinetics (at least three consecutive values of the obtained curve) for each sample was selected to calculate the rate of the reaction. Three citrate synthase samples (two from Isthme Bas, one from Val Studer) and one fumarase sample (from Cataractes) were removed due to debris causing abnormal absorbance curves. The final enzyme activity was corrected by the protein concentration of each sample. Proteins were quantified from five µL of mitochondrial extract reacting with 245µL of Bradford (absorbance red at 595nm), and the protein amounts were calculated from a BSA standard curve.

g) Metabolic phenotyping

Concentrations of metabolites (amino acids, hydroxyl acids, carbohydrates, sugar acids, sterols, aromatics, nucleosides, and amines) were measured from whole body extracts using Gas chromatography – Time of Flight - Mass Spectrometer (GCTOF-MS). From the 75 individuals sampled at the localities of Port Couvreux, Val Travers and Cataractes (formerly colonised habitats), and Pointe Suzanne, Isthme Bas and Val Studer (recently colonised habitats), six males and six females were isolated and vacuum-dried for 15 hours (Speed Vac Concentrator, MiVac, Genevac Ltd., Ipswich, England) before being weighted (Balance XP2U Mettler Toledo, Columbus, OH, d=0.1 µg). The samples were sent to the West Coast Metabolomics Center (University of California, Davis) for whole body extraction and GCTOF-MS procedure, as detailed in Fiehn et al. (2008). Briefly, the samples were extracted through bead-beating (Retsch ball mill, Retsch GbmH, Haan, Germany) for 30s at 25Hz. Then, 1mL of extraction solvent (isopropanol:acetonitrile:water, volume ratio 3:3:2) was added before centrifugation (12 800*g*, 2min, 4 °C). The supernatant was separated into two equal aliquots and vacuum-dried for 4h (Centrivap cold trap, LabCono, Kansas City, MO). Two microliters of a mixture of internal retention index markers, and 10µL of a 20mg.mL^{-1} solution of methoxyamine hydrochloride in pyridine were added to the dried extracts which were further vortexed. Then, a volume of 90µL of N-methyl-N-trimethylsilyltrifluoroacetamide with 1% trimethylchlorosilane was added, and the samples were incubated for 30min at 37 °C. A Gerstel CIS cold injection system (Gerstel GmbH, Müllheim, Germany) provided the injection of 0.5µL of each sample at a rate of 1mL.min^{-1} at an initial temperature of 50 °C, ramping to 250 °C at 12 °C.s^{-1}. Gas chromatography was conducted with an Agilent 6890 equipment, with a 30m column (⌀ 0.25mm); mass spectrometry was conducted with a Leco Pegasus IV. Algorithmic processing of the raw data by ChromaTOF 2.32 software allows to attribute peaks to metabolites, for which quantification is reported as peak height.

h) Statistical analyses

For the analyses, the residence time of the sampled populations was used as a continuous explanatory variable to test for phenotypic responses depending on the population status. Two scales of residence time were used: the experiments that used adult *M. soledadinus* sampled and experimented during the austral summer 2014-2015 were analysed along a colonisation scale ranging from 102 to 3 years (morphometry, starvation resistance, locomotor activity and metabolic phenotyping); the experiments performed on ground beetles sampled during the austral summer 2015-2016 were analysed along a 103 to 4 years old gradient (propensity to disperse, enzymatic assays).

Morphological changes along the invasion gradient

We used the analytical procedure described in Laparie et al. (2013) to describe morphometric variations along the invasion gradient. MANOVAs were used to assess the potential relationship between residence time and size of the measured morphometric traits. In these analyses, the sex was included into the explanatory variables. The normality of residuals was verified with QQplots and Shapiro-Wilk tests. Factorial Discriminant Analyses (*MASS* package, Venables & Ripley, 2002) were performed on separated sex to investigate morphological variations at the intra-population level, and changes among populations. For each sex, a morphological distance matrix was built from the score value of each individual along the first axis of a Canonical Discriminant Analysis (CDA, *candisc* package, Friendly & Fox, 2013). This matrix was then compared with the residence time matrix obtained between each population, using mantel tests (10 000 permutations) in order to quantify trait size modifications.

Starvation resistance

The comparison of starvation resistance among populations was first investigated by computing lethal times (LT_{50} and LT_{90}) with survival regression models (*survival* package, Therneau, 2013). In these models, explanatory variables were sex, residence time and their interaction. Survival duration to starvation was also analysed with linear regression models by considering the number of days each individual survived, as a function of residence time and initial fresh mass. The dynamics of body mass loss until death by inanition was computed as the proportion of fresh mass lost between the first day of the experiment and the last fresh mass recorded for each monitored individual. These data were analysed through linear models, with residence time and sex as the explanatory variables.

Locomotor activity

The substantial dataset resulting from the monitoring of the locomotor activity of adult *M. soledadinus* was first reduced by extracting the number of passages per hour and per population. An increase activity peak was recorded for all individuals between 7h05am and 7h20am, probably caused by the sudden programmed lighting of the room; these periods of 15 minutes were removed from the dataset. Of note, this pattern was not observed when the light was turned off in the evening. The effects of population's residence time and period of the day (simulating daytime and night time) on locomotor

activity were investigated through GLMMs models (the quasi Poisson family was selected because of data over-dispersion).

Dispersal propensity

The proportion of individuals that had left the 'home' box (the individuals in the tube were considered as dispersers) at the end of the experiment was compared using GLMMs (binomial distribution). In this dataset, the dispersal propensity of the six replicates of 10 beetles was compared among the six different populations, with residence time as fixed explanatory variable, and with a random effect of the day of the experiment. A further analysis compared the timing of dispersal, which was calculated as the proportion of individuals that dispersed at each of the three counting checks conducted during the experiment. GLMMs (binomial family) included residence time and the time since the beginning of the experiment (both variables were scaled to Z-scores to enable the convergence of the model), their interaction and the date of the experiment as fixed explanatory variables, added with a random effect of the replicated experimental designs.

Enzymatic assays

The differences in activities in each of the six compounds quantified were investigated using linear regressions, with residence time and sex as explanatory variables.

Metabolic fingerprinting

The GCTOF-MS procedure allowed to quantify 144 metabolites from the 72 samples (6 replicates of two individuals × 6 populations × 2 sexes). To explore this dataset, a Redundancy Analysis (RDA, *vegan* package, Oksanen et al., 2016) was performed on the Z-scaled metabolites' matrix to delineate differences in metabolic phenotypes among populations and sexes. This procedure was selected because, in comparison with discriminant analyses, it allows to include more variables than conditions and it conveniently includes a statistical test. The RDA formula was expressed as follows:

$$Metabolite's\ matrix \sim \log(Residence\ Time)\ x\ Sex$$

Only molecules sharing less than 80% correlation were used in this analysis (124 remaining molecules).

3 - Results

a) *Morphological changes along the invasion gradient*

The eight measured morphological traits significantly differ among specimens sampled along the invasion gradient (approx. $F_{8, 164}$=13.22, P<0.005), and between sexes (approx. $F_{8, 164}$=21.15, P<0.005). There was no interaction between explanatory variables (approx. $F_{8, 164}$=1.47, P=0.17).

The results of the FDA performed for each sex (Fig. 4) depict a morphological gradient along the first axis (46% of the total variance explained for females and 44% for males). On this axis, females are more discriminated than males (inter/within ratio of inertia larger for females than for males). The second axis (31% of the total variance for females, 29% for males) separates 'younger' populations,

composed by individuals of larger sizes, from the individuals collected from older ones, which generally encompass individuals of smaller body size.

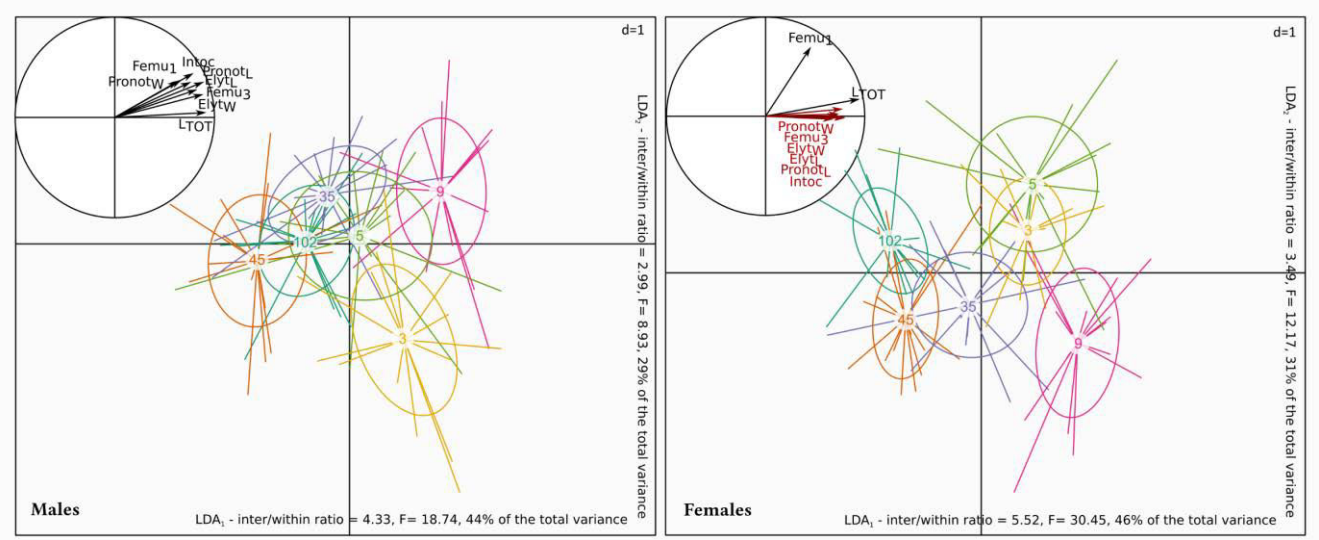

Fig.4 Results of the Factorial Discriminant Analyses performed on the eight morphological traits measured on male and female *M. soledadinus*. Individuals are linked to the centroid of their population, indicated by the residence time. The correlation circles present the projection of the measured variables on the factorial plan. The ratio between inter- and intra-group inertia is provided for the first two axes of each analysis

This result is corroborated by the decreasing canonical scores of individuals along the first axis of the Canonical Discriminant Analyses as a function of increasing residence time (Fig. 5). Mantel tests reveal the significant correlation between the morphological distance matrix and the residence time matrix (females, r=0.46, P<0.001; males: r=0.24, P<0.001). For both sexes, the total body length accounts for a large part of the morphological gradient found, followed, in males, by abdomen width and pronotum size, and, in females, by pronotom size and abdomen size. Interocular distance and femur lengths accounted for a lesser extent to this morphological gradient for both sexes.

b) *Starvation resistance*

The results presented in Fig. 6 show two distinct survival patterns: older populations (Port Couvreux, Val Travers and Cataractes) are characterised by lower abilities to cope with starvation than the younger ones do (Pointe Suzanne, Isthme Bas and Vas Studer). The survival curves were not sorted as a function of the residence times, as outlined by the lethal times presented in Tab. 1.

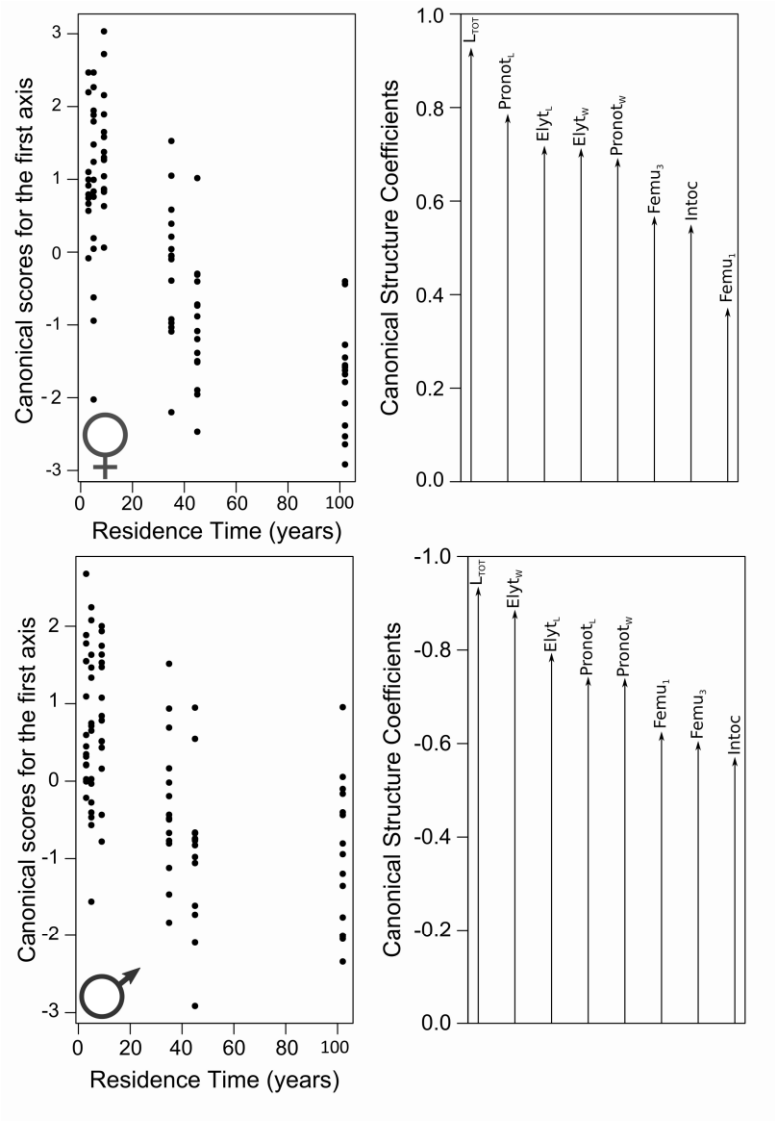

Fig. 5 Results of the Canonical Discriminant Analyses that best depict morphological variations among population of *M. soledadinus*, performed separately on females (upper diagrams) and males (lower diagrams). The score of each individual along the first axis is represented as a function of the residence time of populations. The optimal combination of traits to build the first canonical axis is presented by the canonical structure coefficients

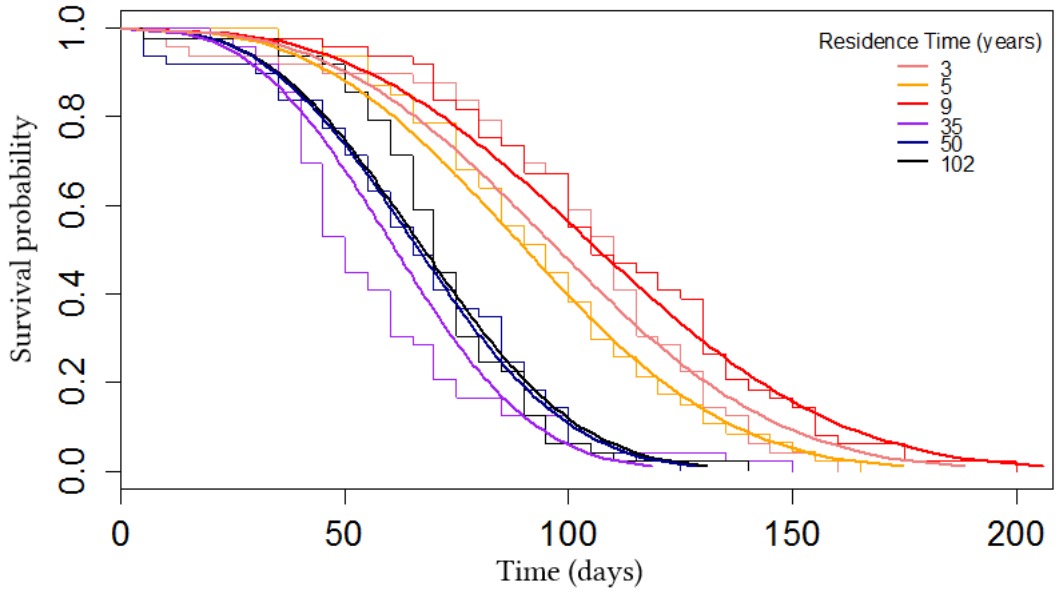

Fig. 6 Survival duration of food deprived adult *M. soledadinus*. For each sampled locality along the invasion gradient, 50 individuals were isolated, maintained at 8°C and supplied with water *ad libitum*. The predicted curve of survival probability is plotted on the real data

Tab. 1 Estimated survival probability (days ±SE) for 50 (LT_{50}) and 90% (LT_{90}) of the populations, depending on the residence time and sex

	Residence Time (years)	LT_{50} (±sd)	LT_{90} (±sd)
Females	102	67.4 ±4.6	104.5 ±7.1
	50	78.2 ±3.2	121.3 ±4.8
	35	81.6 ±3.3	126.6 ±4.9
	9	87.9 ±4.3	136.3 ±6.5
	5	88.9 ±4.5	137.9 ±6.9
	3	89.4 ±4.6	138.7±7.1
Males	102	55.1 ±3.2	85.5±4.9
	50	73.1 ±2.3	113.4 ±3.4
	35	79.3 ±2.3	123.1 ±3.4
	9	91.4 ±3.0	141.8 ±4.5
	5	93.4 ±3.2	144.9 ±4.8
	3	94.4 ±3.4	146.5 ±5.0

Yet, residence time has a significant negative effect on survival regression (z = -3.19, P<0.01). The sex does not affect starvation resistance (z=0.99, P=0.32), but the interaction between residence time and sex is significant (z=-2.25, P<0.05); increased residence time has a stronger negative effect on starvation resistance in males than in females (Fig. 7).

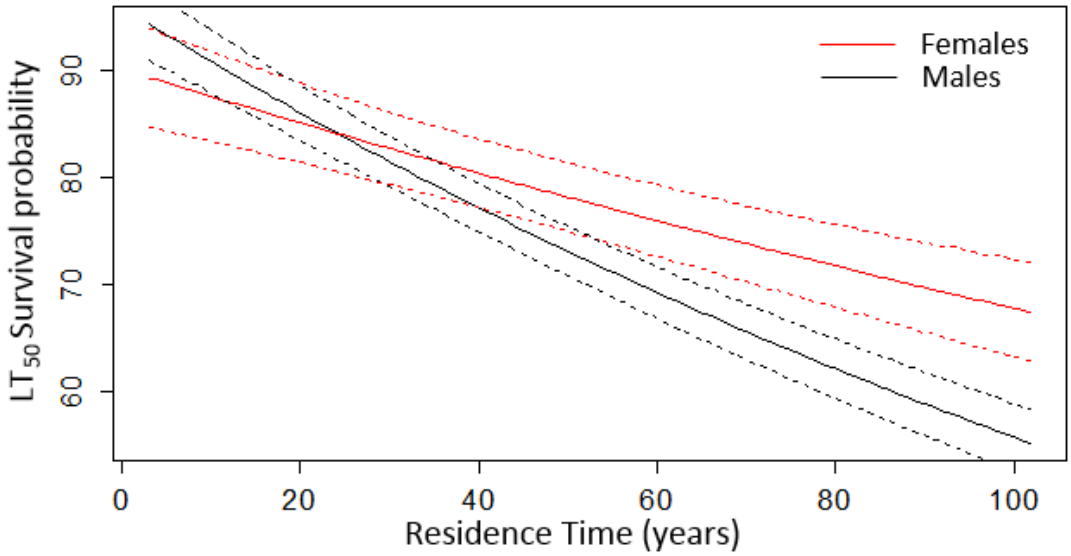

Fig. 7 Logistic regression of the survival probability for 50% (LT_{50}) of males (in black) and females (in red), as a function of residence time. Dotted lines: SE

The same pattern was observed by analysing the number of days before death, proving again that increased residence time correlates with decreased starvation resistance (F=23.37, P<0.001). This effect was more pronounced in males than in females (F=5.09, P<0.05). The initial body mass of the individuals plays a significant role in starvation resistance, with heavier individuals surviving for longer periods of starvation (F=43.49, P<0.001). Residence time does not significantly affects the rate

of mass loss during starvation to death (F=1.98, P=0.16), but females lost significantly more body mass (in proportion) than males (F=41.73, P<0.001), as shown in Fig. 8.

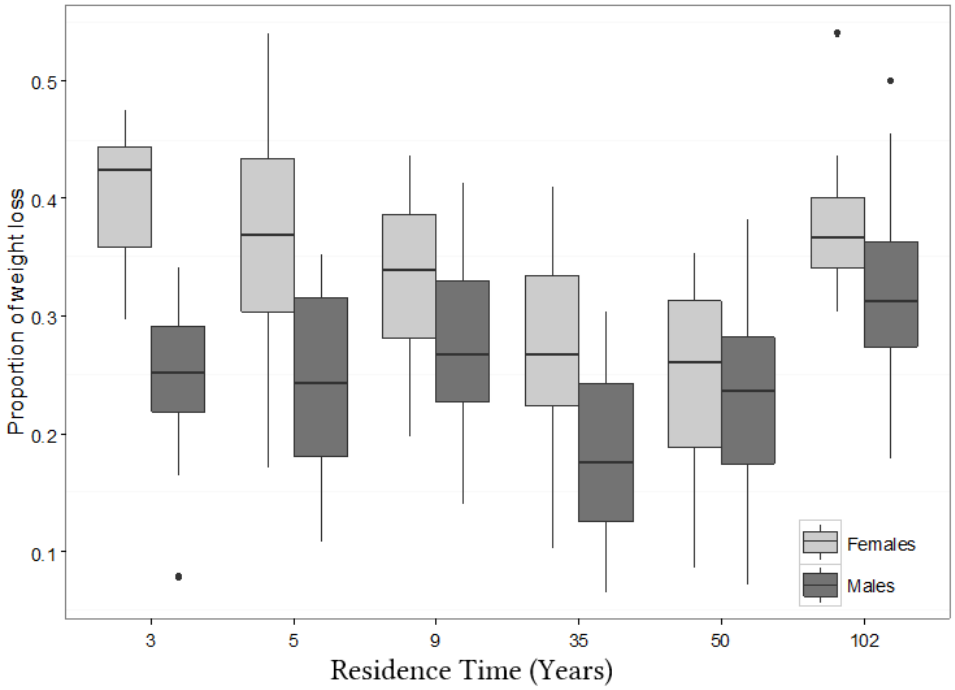

Fig. 8 Proportions of weight lost in individuals (30 per population) during starvation. Each individual was weighted every 5 days from the beginning of the experiment until death. The proportion of weight loss was calculated from the last alive weight recorded – initial weight

c) *Locomotor activity*

This experiment first shows that *M. soledadinus* adults are significantly more active at night (χ^2=71.92, P<0.001) (Fig. 9.a). Increasing residence time has a significant effect on locomotor activity (χ^2=12.45, P<0.001), so that daytime activity of the populations from the invasion front is getting closer and closer to the nocturnal activity of the oldest populations (Fig 9.b).

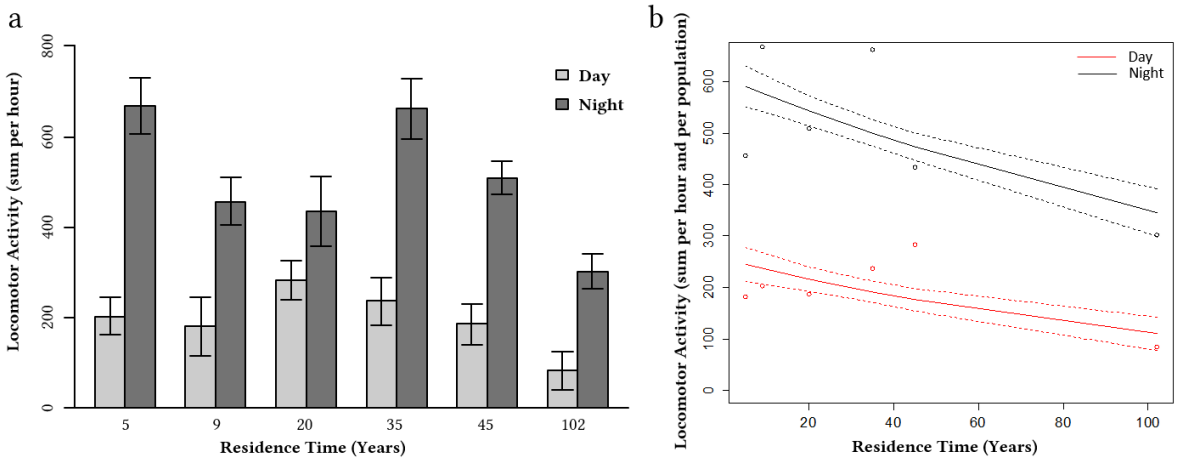

Fig. 9 Comparisons of locomotor activities of adult *M. soledadinus* according to the residence time of their population of origin. (a) Mean (± SE) number of passages per hour recorded for each population (sum per hour of the number of passages of each individual), depending on the lighting conditions of the room (L:D 12:12) simulating daytime and night time. (b) Predicted locomotor activity depending on the residence time and the lighting conditions (empty dots: real data, bold line: quasi Poisson regression, dotted lines: SE)

d) *Dispersal propensity*

The proportion of individuals that dispersed after 36 or 44 hours in the experimental design does not depend on the residence time of the tested populations (χ^2=0.48, P=0.49). In the same way, the timing of dispersal is not different among populations (χ^2=0.18, P=0.67).

e) *Enzymatic assays*

Graphical results of the quantification of antioxidant concentration and enzymatic activities of HK, PGI, citrate synthase, fumarase and malate dehydrogenase are presented in Fig. 10. The statistical results are outlined in Tab. 2. Briefly, sex affects antioxidant concentration (increased in males) and hexokinase activity (increased in females). Residence time has no effect on enzymatic activity, except on phosphoglucoisomerase activity, which is significantly increased in the older tested population.

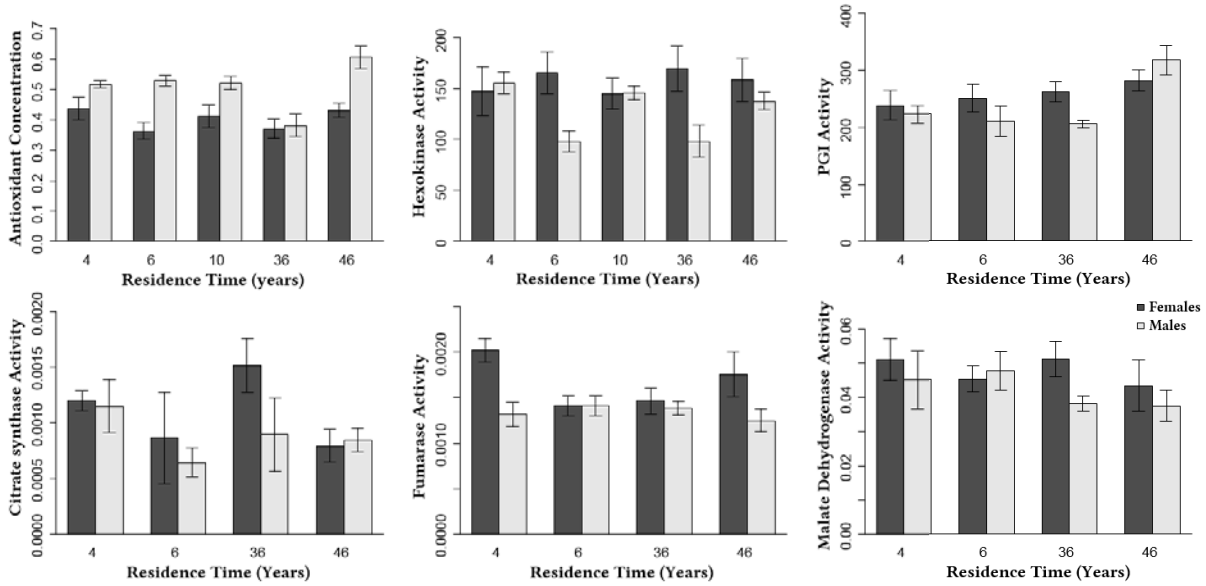

Fig. 10 Quantification (mean ±SE) of each of the six enzymatic activity measured in adult *M. soledadinus* (six samples of two individuals per sex and per population). Total antioxidant activities, phosphoglucoisomerase and hexokinase activities were quantified on whole body extracts, whereas citrate synthase, fumarase and malate dehydrogenase activities were quantified on mitochondrial extracts. All activities are expressed in milliunits.mL.mg^{-1} of fresh mass. Males are represented in light grey and females in dark grey. Corresponding statistical results are outlined in Tab. 2

	Residence Time		Sex	
	χ^2	*P*	χ^2	*P*
Antioxidant	**0.0001**	**0.99**	**21.1**	**<0.001**
HK	**0.0003**	**0.98**	**7.52**	**<0.01**
PGI	**7.52**	**<0.01**	**1.42**	**0.24**
Citrate Synthase	**0.03**	**0.97**	**2.26**	**0.14**
Fumarase	**1.09**	**0.3**	**1.15**	**0.7**
Malate DH	**1.84**	**0.18**	**2.02**	**0.16**

Tab. 2 Statistical results of the linear regressions performed on enzymatic activity measurements. Explanatory variables included residence time and sex

f) *Metabolic fingerprinting*

The RDA performed on the 124 metabolites out of the 144 identified compounds explained 10.41% of the total variance, and depicted significantly different metabolites associations (F_3 = 2.634, P = 0.001; see Fig. 11) according to the residence time of the populations (log-transformed, F = 3.624, P = 0.001), the sex of the replicates (F = 2.553, P = 0.001), and their interaction (F = 1.727, P = 0.035). The first axis, which accounted for 47.31% of the constrained variance, separated males and females, and the second axis (31.26% of the constrained variance) grouped together the samples according to the residence times of the populations, by separating the three formerly established populations (Port Couvreux, Val Travers, Cataractes) from the three recently established populations (Pointe Suzanne, Isthme Bas, Val Studer).

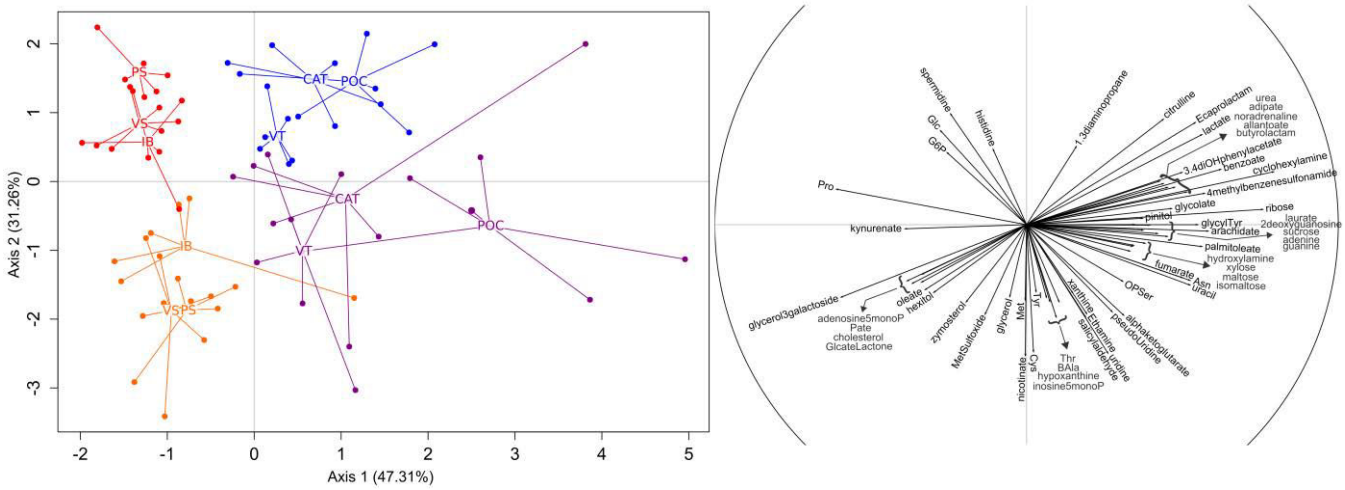

Fig. 11 Result of the RDA performed on the metabolites quantified in *M. soledadinus* sampled in six populations along the invasion gradient. Each replicate (represented by dots, *N*=6 replicates of two beetles per population and per sex) is linked to the centroid of its class (6 population x 2 sexes). Respective to the time since establishment of populations (formerly established populations: Port Couvreux POC, Val Travers VT, Cataractes CAT; recently established populations: Pointe Suzanne PS, Isthme Bas IB, Val Studer VS), the females are in purple and orange, the males are in blue and red. The proportion of the constrained variance explained by each axis is provided within brackets. The correlation circle depicts the normed reaction (from -1 to 1) between the quantified metabolites and the two axes of the factorial plan. For readability purposes, only metabolites with a correlation coefficient > |0.3| are shown (N = 62 compounds). See Supplementary Table 1 for complete list of metabolites included in the analysis and corresponding correlation coefficients

4 - Discussion

Our results report some of the phenotypic changes occurring in adult *M. soledadinus* sampled along its invasion gradient at the Kerguelen Islands. Populations with larger residence times showed a significant reduction in body size, starvation tolerance, locomotor activity, concentrations in phosphoglucoisomerase and metabolic fingerprints of the individuals. Compared to formerly established populations, populations with lower residence time host larger individuals, characterized by better resistance to food deprivation and increased locomotor activity. In parallel, individuals have lower amounts of phosphoglucoisomerase and generally a different set of free-circulating metabolites. No difference was found in the propensity to disperse, and, from a physiological standpoint, activities

of five enzymes of major importance in glycolysis and Krebs cycle were comparable. Sex was also an important factor explaining differences between individuals.

The morphometric analysis confirms the morphological gradient described earlier by Laparie et al. (2010, 2013). As our measurements were performed on different populations (except Port Couvreux and Port Elizabeth that are included in these three studies) sampled at different time points, this finding gives a high level of confidence to this body size gradient. Individuals from the invasion front are significantly larger than individuals from formerly established (core) populations. This larger size is predominantly explained by increased size of different tagma depending on sex: females have larger pronotum size and males have larger abdomen size. Variations in the accessibility to trophic resources among localities may contribute to this body size pattern. Lebouvier et al. (2011) pointed out the existence of a negative correlation between the abundances of native species and *M. soledadinus* in formerly colonised localities. The predation pressure of *M. soledadinus* larvae and adults on potential preys could impose shifts towards preying on invertebrates of suboptimal quality in core populations. Conversely, at the invasion front, colonists would benefit from a larger - potentially unlimited - pool of preferred preys, authorizing the production of offspring of larger sizes. However, this hypothesis cannot solely explain this body size pattern, which has also been demonstrated in insects from other taxa (Hill et al., 1999; Abril et al., 2013).

In our model system, individuals of *M. soledadinus* from Cataractes (36 years of residence time) are among the smallest measured insects. At this locality, prey diversity and density are still relatively important. For instance, there are large populations of native diptera (pers. obs.), which are profitable for *M. soledadinus* (Laparie et al., 2012). Conversely, the individuals from Val Studer (range edge population, 4 years of residence time) have large body size despite low estimated prey availability/diversity in this inland valley. Altogether, these observations suggest that there may be other important factors triggering this morphological gradient, of which spatial sorting at the expanding range of *M. soledadinus* could be proposed.

Larger individuals can reach more distant localities during colonisation events, as a result of complex interconnections and interactions among ecological, morphological and biological characteristics. Specifically (non-exhaustive list), longer legs, higher muscle mass, larger body reserves, lower competition in newly colonised habitats should enhance dispersal performance, and should increase the probability of dispersal events over higher geographic distances. This process repeats as the invasion wave progresses, thus reinforcing the phenotypic distinction between front (disperser) and core (resident) populations. Moreover, competitive ability and energetic allocation also play a central role in setting body size patterns along invasion gradients. Core populations are often close to their carrying capacity, meaning that intra- and inter-specific competition is often high. In these conditions, resource allocation to competitive ability must be increased, in addition to the laying of a higher amount of eggs of smaller sizes by females (bet hedging strategy should be promoted, together with higher egg production, as the hatching probability is assumed to decline in core

populations, at least for predatory species such as *M. soledadinus*). Performance of dispersal, reproduction and competitive ability has been suggested to significantly interconnect (Travis et al., 2009), and this is even truer for the last two biological traits. As intraspecific competition is highly reduced at the invasion front, a higher investment towards reproduction can be expected. This higher reproductive investment should be accompanied by the laying of a small amount of eggs of bigger sizes, as the hatching rate is supposedly higher at the invasion front.

The behavioural, physiological and biochemical changes associated with morphological differences along invasion gradients remain largely unexplored in entomological studies. Several authors suggested the existence of different phenotypes among core and range populations (Lyytinen et al., 2009; Laparie et al., 2013; Lombaert et al., 2014; Therry et al., 2015), but the association of traits, altogether forming a syndrome of dispersal in front populations remain non-elucidated. The large-scale phenotyping study we conducted showed that, in controlled conditions, adults from range margin populations exhibit higher locomotor activity. Increased movement performance is an explicit advantage for colonising individuals. However, this advantage can be significant in successfully reaching more distant locations, only if individuals disperse using straight trajectories (Conradt & Roper, 2006; Delattre et al., 2010). Displacement patterns could then be investigated to further explore dispersal behaviour of adult *M. soledadinus*.

As a complement of the locomotor activity, we studied another dispersal-related behavioural trait, by assessing the propensity of individuals to leave a patch. No difference was found between populations, which could mean that the same proportion of disperser/resident insects occur within the tested populations. This experimental design could be further use to sort out individuals according to their behaviour. Indeed, bolder adult *M. soledadinus* could have leave the pipe quicker, whereas shy individuals stayed longer into the patch. Because experiments were only conducted at one single density (10 individuals per patch), it is possible that individuals behave as trendy lemmings in this experiment, following explorers. We must thus acknowledge that this study, instead of comparing dispersal propensity, may have more likely tested for behavioural sorting (Cote et al., 2010).

Dispersal is a costly behaviour, in terms of risk undertaken and energetic investment (see Bonte et al., 2012 for a review). Front populations host larger individuals of *M. soledadinus*, and this has been assumed to increase the amount of body stores, in turn allowing these insects to withstand longer periods of starvation and/or better cope with abiotic stressors (Renault et al., 2003). Indeed, even if dispersal is costly, the interruption of reproduction during this period can balance this cost, and thus, an individual having larger body reserves have greater chances to cover longer geographic distances and successfully reach another habitat patch. This assumption should be particularly relevant in landscapes where favourable patches are fragmented. Here, we compared starvation resistance of insects, as well as temporal dynamic of mass loss during food deprivation, among insects sampled from core populations and those sampled from edge populations. Survival durations of insects

from range edge populations was significantly increased compared with insects from core populations, and the ability to cope with starvation was generally enhanced in heavier individuals. According to our results, males gained along the invasion gradient comparatively more resistance to starvation than females. Increased body size in males from recently established populations, was primarily influenced by the size of their abdomen. The larger amount of body reserves that could be stored if abdomen size are larger could enhance resistance to starvation. Nevertheless, this could only be efficient if the global energetic needs of these larger insects are lower, or at worst equivalent, than those from small insects.

The increased performance to cope with food deprivation in adult *M. soledadinus* sampled in recently colonised localities should be assisted by adjustments of metabolic activities, with enzyme activity oriented towards a higher production of storage reserves. The total energy diverted to the different functional traits of organisms generates trade-offs (Cody & Overton, 1996). Consistently, Harrison et al. (2006) highlighted the importance of physiological trade-offs in determining dispersal and range limits of invasive honeybees. We did not investigated the performance of would other energy-consuming life traits (somatic maintenance, competitive ability, immunity, mating…), and did not quantify and compared among populations the costs of reproduction and dispersal. We rather focused on biochemical correlates, by comparative assessments of activities of enzymes of glycolytic and tricarboxylic cycle pathways. The most significant difference was measured between males and females, which may probably result from the higher energy-demanding costs of reproduction in females. Meanwhile, we did not observe stringent differences for the activity of these enzymes in the considered populations of *M. soledadinus*. Earlier studies on dispersal ecology reported that PGI can be used as a convenient proxy for predicting the dispersal performance of the individuals. This enzyme received intensive focus in invasive species due to its implication in dispersal activity (Niitepõld, 2010; Mitikka & Hanski, 2010). In this study, PGI showed a correlation with residence time, with increased activity in the sampled from the oldest population tested. This pattern may be related to the lower energetic costs of dispersal in this non-flying carabid beetle. The similar enzyme activity does suggest that the potential for energy production by the main core of the energetic metabolism is not altered along the invasion gradient. This finding nicely supports the idea of energetic constraints that must be differentially balanced among the different body compartments according to the ecological conditions experienced by the insect. These data also give strong support to the importance of physiological plasticity as a driver of evolutionary responses in insects (Agrawal, 2001; Chown et al., 2007).

Phenotyping of adult *M. soledadinus* sampled from populations distributed along the invasion gradient highlights similarities in the activity of the primary metabolism (enzymatic activities). All populations are close at the Kerguelen Islands, both genetically (unique introduction event a century ago, see **Chapter 2**) and geographically, and they were sampled in similar microhabitats to smooth as much as possible the effects of micro-environmental variations. Our results demonstrate that energy can be produced in a similar fashion among populations along the invasion gradient.

At a finer scale however, metabolomic investigations show that populations were characterised by distinct metabolic phenotypes depending on the sex first and residence times of the populations. The results seems to indicate a greater inter-replicate/intra-population variations in females than males. This may be explained by differences in the reproductive status of the samples, as the presence and number of eggs can greatly modify the contents in proteins and lipids of whole-body extracts. Population of similar residence time (more than 35 or less than 10 years since establishment) are grouped together in the analysis, indicating general distinct metabolic phenotypes of populations at the invasion front compared with formerly established populations.

This preliminary conclusion is different from the work of Tracy et al. (2012). These authors described the spatial sorting of *Rhinella marina,* which displays distinct morphological and behavioural patterns along the invasion wave, without physiological distinctions highlighted in the different dispersal-related traits investigated. However, specific investigations are required to further interpret this metabolomics dataset. The next step consists in delineating specific metabolic pathways that could be different between populations according to their residence time.

The amount of results provided by this study highlight that distinct phenotypic traits are affected by residence time of populations, demonstrating the existence of spatial sorting. Fine scale metabolic inquiries are required to help understanding the underlying mechanisms of phenotypes differentiation, as well as to elucidate physiological trade-offs favouring dispersal at the invasion front. Physiological adjustments to environmental variables occur at several levels of organisation, all being sustained by the metabolic network (production of energy and primary and secondary metabolites which are important bricks for physiological remodelling). Increased energetic demand associated to elevated performance of one trait modifies global metabolic network operations (van Petegem et al., 2016). In further analyses, more in-depth data computation should be developed and used (Cottret et al., 2010), in order to draw metabolic stories by connecting the metabolites that best explain groups separation. We do believe that this is the only way for elaborating the role of these metabolites within each of the proposed invasion gradient scenario.

Acknowledgments

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Supplementary Material

Tab.1 Summary of the 124 metabolites included in the RDA analysis and corresponding correlation coefficients with each axes of the analysis (RDA_1 and RDA_2). The first column echoes the metabolite's names used in the correlation circle of the Fig. 11, of which full names and KEGG numbers are displayed in the second and third columns. The bold entries correspond to metabolites plotted in the correlation circle.

Metabolite's denomination	Name	KEGG	RDA_1	RDA_2
1.3diaminopropane	**1,3-diaminopropane**	**C00986**	**0.166**	**0.184**
1monoolein	1-monoolein		-0.063	-0.129
1monopalmitin	1-monopalmitin	C01885	-0.056	0.019
2deoxyguanosine	**2'-deoxyguanosine**	**C00330**	**0.203**	**-0.010**
2ketoisocaproicAcid	2-ketoisocaproic acid	C00233	0.081	-0.101
2monoolein	2-monoolein		-0.264	-0.014
2OHglutarate	2-hydroxyglutaric acid	C02630	0.163	0.157
3.4diOHphenylacetate	**3,4-dihydroxyphenylacetic acid**	**C01161**	**0.275**	**0.119**
4aminobutyricAcid	4-aminobutyric acid	C00334	0.136	0.027
4methylbenzenesulfonamide	**4-methylbenzenesulfonamide**	**C14412**	**0.269**	**0.039**
5decanoate	pentadecanoic acid	C16537	-0.022	0.086
9decanoate	nonadecanoic acid	C16535	-0.021	-0.090
adenine	**adenine**	**C00147**	**0.289**	**-0.097**
adenosine	adenosine	C00212	0.060	-0.153
adenosine5monoP	**adenosine-5-monophosphate**	**C00020**	**-0.252**	**-0.203**
adipate	**adipic acid**	**C06104**	**0.176**	**0.157**
adrenaline	adrenaline		0.031	0.066
Ala	alanine	C00041	-0.095	-0.041
AlaAla	alanine-alanine		0.202	-0.117
allantoate	**allantoic acid**	**C00499**	**0.273**	**-0.048**
alphaketoglutarate	**alpha-ketoglutarate**	**C00026**	**0.131**	**-0.240**
aminomalonate	aminomalonate	C00872	0.046	-0.133
arachidate	**arachidic acid**	**C06425**	**0.327**	**0.035**
arachidonate	arachidonic acid	C00219	-0.028	-0.210
Asn	**asparagine**	**C00152**	**0.290**	**-0.206**
BAla	**beta-alanine**	**C00099**	**0.154**	**-0.235**
benzoate	**benzoic acid**	**C00180**	**0.344**	**0.122**
butyrolactam	**butyrolactam NIST**		**0.290**	**0.128**
cholesterol	**cholesterol**	**C00187**	**-0.206**	**-0.154**
citrate	citric acid	C00158	0.015	-0.031
citrulline	**citrulline**	**C00327**	**0.163**	**0.247**
conduritolBepoxide	conduritol-beta-epoxide		-0.086	-0.164
cyclohexylamine	**cyclohexylamine**	**C00571**	**0.457**	**0.079**
Cys	**cysteine**	**C00097**	**-0.099**	**-0.392**
cystine	cystine	C01420	0.091	-0.112
Ecaprolactam	**epsilon-caprolactam**	**C06593**	**0.334**	**0.212**
epicatechin	epicatechin	C09727	0.075	0.114
Ethamine	**ethanolamine**	**C00189**	**0.162**	**-0.256**
EthP	ethanol phosphate NIST		-0.190	-0.130
fructose	fructose	C02336	0.137	-0.061
fumarate	**fumaric acid**	**C00122**	**0.248**	**-0.169**
G6P	**glucose-6-phosphate**	**C00092**	**-0.304**	**0.224**
Glc	**glucose**	**C00221**	**-0.233**	**0.244**
GlcateLactone	**gluconic acid lactone**	**C00198**	**-0.281**	**-0.192**
Gln	glutamine	C00064	0.013	-0.074
Glu	glutamic acid	C00025	0.071	0.176
glycerate	glyceric acid	C00258	0.158	-0.065
glycerol	**glycerol**	**C00116**	**0.010**	**-0.181**
glycerol3galactoside	**glycerol-3-galactoside**	**C05401**	**-0.412**	**-0.280**
glycolate	**glycolic acid**	**C00160**	**0.205**	**0.114**
glycylPro	glycyl-proline		0.157	-0.061
glycylTyr	**glycyl tyrosine**		**0.325**	**-0.133**

guanine	**guanine**	**C00242**	**0.195**	**-0.035**
guanosine	guanosine	C00387	0.272	-0.160
hexitol	**hexitol**	**C00392**	**-0.197**	**-0.370**
hexose	hexose NIST		-0.207	0.050
hexuronate	hexuronic acid		-0.073	0.049
histidine	**histidine**	**C00135**	**-0.099**	**0.189**
hydroxylamine	**hydroxylamine**	**C00192**	**0.091**	**-0.077**
hypoxanthine	**hypoxanthine**	**C00262**	**0.113**	**-0.193**
indole3lactate	indole-3-lactate	C02043	-0.078	0.009
inosine	inosine	C00294	0.158	-0.136
inosine5monoP	**inosine-5'-monophosphate**	**C00130**	**0.099**	**-0.313**
inositol4monoP	inositol-4-monophosphate	C03546	-0.085	0.084
iso7decanoate	isoheptadecanoic acid NIST		0.041	0.002
isomaltose	**isomaltose**		**0.048**	**-0.101**
kynurenate	**kynurenic acid**		**-0.324**	**0.046**
lactate	**lactic acid**	**C01432**	**0.370**	**0.215**
laurate	**lauric acid**	**C02679**	**0.188**	**-0.004**
linolenate	linolenic acid	C06427	-0.268	-0.140
Lys	lysine	C00047	0.170	-0.021
malate	malic acid	C00711	-0.131	-0.158
maleimide	maleimide	C07272	0.227	-0.075
maltose	**maltose**	**C00208**	**0.212**	**-0.150**
Met	**methionine**	**C00073**	**0.012**	**-0.360**
methgalactose	1-methylgalactose NIST		-0.052	-0.183
mEthP	methanolphosphate		-0.036	-0.157
MetSulfoxide	**methionine sulfoxide**	**C02989**	**-0.074**	**-0.202**
myoInositol	myo-inositol	C00137	-0.168	0.116
NacetylDgalactosamine	N-acetyl-D-galactosamine		0.166	-0.100
NacetylDhexosamine	n-acetyl-d-hexosamine	C03878	-0.096	0.023
Nacetylglcamine	UDP-N-acetylglucosamine	C00043	0.015	-0.008
nicotinate	**nicotinic acid**	**C00253**	**0.037**	**-0.420**
NmethylAla	N-methylalanine	C02721	-0.337	-0.213
noradrenaline	**noradrenaline**		**0.217**	**0.085**
OHcarbamate	hydroxycarbamate NIST		0.086	0.071
oleate	**oleic acid**	**C00712**	**-0.205**	**-0.188**
OPSer	**O-phosphoserine**	**C01005**	**0.067**	**-0.133**
ornithine	ornithine	C00077	0.188	-0.103
oxoPro	oxoproline	C01879	0.127	-0.093
palmitoleate	**palmitoleic acid**	**C08362**	**0.253**	**-0.079**
parabanate	parabanic acid NIST	C00802	0.201	0.183
Pate	**phosphate**	**C00009**	**-0.118**	**-0.187**
PEthamine	phosphoethanolamine	C00346	0.054	-0.030
phenylacetamide	phenylacetamide	C02505	-0.120	0.145
pinitol	**pinitol**	**C03844**	**0.184**	**-0.054**
pipecolinate	pipecolinic acid		-0.195	0.088
Pro	**proline**	**C00148**	**-0.345**	**0.123**
pseudoUridine	**pseudo uridine**	**C02067**	**0.235**	**-0.171**
putrescine	putrescine	C00138	0.008	-0.057
pyruvate	pyruvic acid	C00022	0.014	-0.248
ribitol	ribitol	C00474	0.054	0.094
ribonate	ribonic acid	C01685	0.156	-0.020
ribose	ribose	C00121	0.439	0.010
salicylaldehyde	**salicylaldehyde**	**C06202**	**0.148**	**-0.245**
Ser	serine	C00065	-0.033	-0.047
sorbitol	sorbitol	C00794	0.132	0.008
spermidine	**spermidine**	**C00315**	**-0.325**	**0.310**
succinate	succinic acid	C00042	0.307	0.248
sucrose	**sucrose**	**C00089**	**0.078**	**-0.043**
taurine	taurine	C00245	0.049	0.190
Thr	**threonine**	**C00188**	**0.151**	**-0.150**
thymidine	thymidine	C00214	0.107	0.020
thymine	thymine	C00178	0.107	-0.076
trehalose	trehalose		-0.025	-0.004

Trp	tryptophan	C00078	-0.167	0.131
Tyr	**tyrosine**	**C00082**	**0.057**	**-0.154**
uracil	**uracil**	**C00106**	**0.259**	**-0.144**
urate	uric acid	C00366	0.152	-0.067
urea	**urea**	**C00086**	**0.248**	**-0.010**
uridine	**uridine**	**C00299**	**0.226**	**-0.268**
xanthine	**xanthine**	**C00385**	**0.177**	**-0.113**
xylose	**xylose**	**C00181**	**0.144**	**-0.081**
zymosterol	**zymosterol**		**-0.269**	**-0.210**

Chapter 4

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The importance of relative humidity and trophic resources in governing ecological niche of the invasive carabid beetle *Merizodus soledadinus* in the Kerguelen archipelago

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Highlights

- At the Kerguelen Islands *M. soledadinus* is distributed along seashores and watercourses
- In order to understand the effects of trophic resources and water availability on spatial distribution, individuals were exposed to food and water stress. Survival duration and metabolic changes were analysed
- Results show that *M. soledadinus* is strongly affected by water stress, suggesting that relative humidity and water availability dictate the realized niche of this species at the Kerguelen Islands

1 - Introduction

Human-mediated invasive species distribution and dispersal have received growing interest over the past few decades, largely due to the significant impact of invasive species on ecosystem services, on the economy (Cook et al., 2007; Pejchar & Mooney, 2009), and on biodiversity (Cassey et al., 2005). In several cases, it remains unclear whether invasive species are the main drivers of change in community assemblages and ecosystem functioning, passengers benefiting from the ecological alterations of disturbed habitats, or a combination of these two factors (MacDougall & Turkington, 2005). Regardless of the circumstances, the main environmental parameters governing establishment success and subsequent range expansion are case-dependent (Zenni & Nuñez, 2013; Ehrlén & Morris, 2015), most likely due to species-specific survival, reproduction, growth, and recruitment factors. Interestingly, although current predictions of the changing ranges of non-native taxa are examined with indirect approaches (e.g. species distribution models), the environmental and mechanistic factors driving geographical range expansion are seldom studied directly. For ectothermic species, temperature, and its variation at different temporal scales (Colinet et al., 2015), trophic resource availability (Romanuk & Kolasa, 2005), and/or relative humidity (RH) and water availability may be pivotal in establishing each species' fundamental realised niche.

Demography and dispersal are driven by stage- and size-specific physiological requirements during invasion process. For instance, water availability can shape the distribution of insect species, be they invasive or native (Hoffmann et al., 2003). Decreased water availability, even if it is minor, can hamper insect survival, particularly for polar species (Everatt et al., 2014). In addition to water availability, energy input, critical for sustaining reproduction, growth, development, and vital biological function is often a strong predictor of species distribution and population density (Hawkins et al., 2003). Variation in these abiotic factors can push insects outside their physiological tolerance window, particularly during range expansion. In fact, terrestrial insects may encounter food and water stress during dispersal events. Intensity of these stressors can be biologically significant when non-flying insects must cover high dispersal distances, particularly when the distribution of feeding and drinking areas is patchy.

Insects often encounter restrictive conditions in their (micro)habitats. A range of physiological adaptations to overcome these environmental constraints has been observed in insects (Teets & Denlinger, 2013). Yet, responses mounted by food-deprived insects during active dispersal are relatively limited and roughly consist of anticipatory augmentation in the amount of body stores and/or reduction of energetic demands (Rion & Kawecki, 2007). Alternatively, bodily water loss can be counteracted by a broader response arsenal (Benoit, 2010; Teets et al., 2012), including accumulation of osmolytes (polyols, sugars, and amino acids), which has often been reported in desiccation-stressed insects (Crowe et al., 1992; Yancey, 2005). Physiological changes elicited by desiccating conditions can be monitored conveniently by metabolomics analyses (Foucreau et al., 2012; Hidalgo et al., 2013),

which often depict divergent metabolic phenotypes between water-stressed and control insects (Hidalgo et al., 2014). The flightless ground beetle *Merizodus soledadinus*, which is invading Îles Kerguelen, is no exception (Hidalgo et al., 2014). This species establishes moderate to dense populations in coastal areas in this archipelago (Lebouvier et al., 2011), where it thrives under seaweed along the foreshore and is thus exposed to osmotically challenging conditions

A recent field-based study of the habitat distribution of *M. soledadinus* in the Kerguelen archipelago emphasised the importance of microhabitat moisture levels in the species' distribution (Renault et al., 2015). This finding was consistent with the pioneer studies of Ottesen (1990) and Todd & Block (1997) on desiccation sensitivity in *M. soledadinus* adults. Furthermore, prey dependency in *M. soledadinus* could represent an alternative hypothesis for its distribution pattern. Most of the prey consumed by this carabid beetle, in the form of fly larvae, is distributed along coastal habitats. Finally, the abiotic factors discussed above might constrain main dispersal paths used by *M. soledadinus*, given that large rocky plateaus often separate potential trophic patches at Îles Kerguelen. This information might be particularly relevant to our predictions for the future range expansion and impacts of *M. soledadinus*, which became largely dispersed over several decades in the Kerguelen archipelago in the absence of human assistance.

In the present study, we evaluated the importance of habitat moisture and the availability of trophic resources in the geographic range establishment of *M. soledadinus* in the Kerguelen archipelago. This question was addressed by examining the capability of adult *M. soledadinus* to tolerate food and water stress. First, we determined the species resistance level to food deprivation by starving adult individuals to death. Second, we assessed water-deprived adults' capacity to withstand weak, moderate, or extreme desiccating conditions. The following hypotheses were tested: (i) *M. soledadinus* will resist starvation for several weeks if adults are supplied with water *ad libitum*; (ii) survival time will be strongly reduced if RH is decreased; (iii) osmolytes in the form of amino acids and polyols will accumulate to combat desiccation-induced reduction of body water content. Because *Merizodus soledadinus* is wingless, the presently sought information is indispensable to infer its main colonisation routes at Îles Kerguelen.

2 - Methods

a) *Insect distribution, sampling, rearing conditions, and experimental design*

All *M. soledadinus* records at Îles Kerguelen have been georeferenced annually since 2005 according to a standardised index. Each observation period was fixed to 10 min per person, resulting in 1695 records being indexed in a database. This information was extracted for plotting the current *M. soledadinus* geographic range at Îles Kerguelen (Fig. S1).

Four hundred *M. soledadinus* adults were hand-collected under rocks in December 2013 at Port-aux-Français (Îles Kerguelen; 49°21'S, 70°13'E; mean annual temperature ~ 5 °C; Fig. S1). The live specimens were maintained for three months under dark conditions at a temperature of 5-6 °C in four

plastic boxes (17.9 × 12 × 7.4 cm) on moist sand (85-95% RH) and fed *ad libitum* with dipteran maggots. Subsequently, *M. soledadinus* adults were transferred from 5-6 °C to 8 °C (mean temperature during summer months; source: Météo France) and maintained at this temperature for one week. Following one week at 8 °C, the same adults were kept at 15 °C (highest summer daytime temperature reached in the archipelago; source: Météo France). The adult individuals were maintained at this highest temperature for two weeks prior to being subjected to experiments. For all thermal conditions, the insects were kept on moist sand and fed with *Calliphoridae* larvae *ad libitum* (light/darkness: 12:12, RH: 85-95%).

b) *Starvation resistance*

Thirty adult *M. soledadinus* individuals (sex ratio 1: 1) were used to test survivorship under starvation conditions. *Merizodus soledadinus* beetles were placed in Petri dishes (ø = 8.5 cm) with the bottom covered by filter paper, and maintained at 8 °C (light/darkness: 12:12, RH: 85-95%). Each adult was placed individually in a Petri dish to avoid cannibalism and was supplied with water (an Eppendorf® microtube filled with water and stoppered with a cotton ball). Survival and water availability were checked every two days until the death of all individuals.

c) *Desiccation resistance*

Adult beetles were exposed to three RH conditions: 100%, 70%, and 30%. These conditions were established by filling the bottom of rectangular plastic boxes (17.9 × 12 × 7.4 cm) with 3 cm of (i) water (100% RH condition), (ii) 50% diluted $CaCl_2$ (70% RH), or (iii) saturated $CaCl_2$ (30% RH). Five circular plastic containers (Ø: 8.5 cm; height: 4.7 cm), each with an absorbent disc positioned at the bottom of the container, were positioned on the liquid layer for each experimental condition (i, ii, iii). Ten adult *M. soledadinus* beetles were transferred to each container, and the rectangular boxes were sealed hermetically with tape. The beetles were maintained at 15 °C and were food- and water-deprived during the entire experiment. Two sets of rectangular boxes were designed, one for survival assays and one for metabolomics assays.

A preliminary experiment was conducted at the three RH conditions over a 12 h period, with carabid beetle survival assayed every 2 h. This exposure duration was not long enough to result in 100% mortality under the most stressful condition (30% RH, 15 °C); therefore a second survival assay was conducted over a 2-d period. Fifty individuals were maintained at 100% RH at 15 °C until all of them died. Survival was scored as the number of individuals moving in the circular boxes; beetles lying on their backs and not responding to stimuli were considered dead. RH was monitored inside the rectangular boxes using iButton humidity/temperature logger (1-Wire Hygrochron DS1923, Maxim Integrated, San Jose, USA) and was 96.4 ± 0.9% (100% RH), 70.3 ± 0.4% (70% RH), and 35.3 ± 3.9% (30% RH). We did not observe cannibalism during these experiments.

Next, our objective was to compile and compare our survival results with Todd and Block (1997). An additional survival result was included in this analysis: adult *M. soledadinus* were deprived

of water and food, and exposed to 8 °C and 75% RH conditions until death (Renault, Hotte, Ouisse, unpublished data). Experimental conditions (temperature, RH) from all studies were converted to a single variable, vapour pressure deficit (VPD) to perform direct comparisons. For all data, VPD was calculated following Pappas et al. (2008) formula:

VPD (kPa) = ((1-[RH/100])*saturated vapour pressure)/100

All details are provided in Table S1. Finally, one iButton humidity/temperature logger (1-Wire Hygrochron DS1923, Maxim Integrated, San Jose, USA) was positioned under a stone at Port-aux-Français from the 11th through the 18th of December, 2015 to monitor temperature and RH in the microhabitat (Table S2).

d) *Body water and metabolic fingerprinting*

Exposure under different relative humidity conditions

The physiological plasticity of adult *M. soledadinus* facing desiccation was evaluated by monitoring beetle water content and metabolic variation over the exposure duration under the three experimental conditions. When sampled, the specimens were plunged directly into liquid nitrogen at T_0 (control), T_4, T_8, and T_{16}, *i.e.* after 0 h, 4 h, 8 h, or 16 h of exposure at 100%, 70% or 30% RH (N = 6 replicates of two pooled individuals for each experimental treatment). Ninety percent of adult *M. soledadinus* survived until T_8 at 30% RH, and then died rapidly under this experimental condition; it was not possible to run analyses at T_{16} for this experimental condition. Subsequently, the samples were stored on ice and weighed rapidly (fresh mass, mg). Afterwards, *M. soledadinus* samples were lyophilised for 96 h before being reweighed (dry mass, mg). Body water amount was computed as follows: body water = fresh mass - dry mass. The samples were stored at 20 °C prior to further processing. Metabolic fingerprints were obtained from whole body extracts (N = 6 replicates per experimental condition, each replicate being made of two pooled unsexed adults).

Sample preparation and derivatisation

Concentrations of non-structural carbohydrates, organic compounds, and amino acids were measured using gas chromatography/mass spectrometry (GC-MS, Thermo Fisher) following Khodayari et al. (2013). Methanol-chloroform (600 µL, ratio 2: 1) was added to each sample, each of which was homogenised with a bead-beating device at 25 Hz for 90 s. Four hundred microlitres of ultrapure water was added to each sample, which were further centrifuged for 10 min at 4000 *g* at 4 °C. Ninety microlitre aliquots of supernatant containing polar metabolites were transferred to microtubes and vacuum-dried. These aliquots were re-suspended in 30 µL of 20 mg mL^{-1} methoxyaminehydrochloride (Sigma-Aldrich, St. Louis, MO, USA) in pyridine prior to being incubated under orbital shaking at 40 °C for 60 min. Following incubation, 30 µL of N-methyl-N-(trimethylsilyl) trifluoroacetamine (Sigma, #394866) was added to each aliquot and derivatisation was conducted at 40 °C for 60 min under agitation. Calibration curves were generated and used to quantify metabolite

concentrations; samples consisting of 60 pure reference compounds were run at concentrations of 1 µM, 2 µM, 5 µM, 10 µM, 20 µM, 50 µM, 100 µM, 200 µM, 500 µM, 750 µM, 1000 µM, and 1500 µM.

e) *Statistical analyses*

We computed lethal times for 50% (LT_{50}) and 90% (LT_{90}) of adult *M. soledadinus* for the survival experiments using survival regression models (*survival* package; Therneau, 2013). RH and gender were used as explanatory variables for desiccation and starvation experiments, respectively.

Changes in body water content over the experiment duration were compared across groups exposed to the three experimental conditions (RH 100%, 70%, and 30%) with a linear regression model followed by Tukey's *post hoc* comparisons, with the following formula: relative water content ~ RH × exposure time + dry mass.

The GC-MS procedure facilitated the identification of 48 metabolic compounds from distinct metabolite families: amino acids, carboxylic acids, cyclitols, polyamines, polyhydroxy acids, polyols, and sugars. Following homogenisation of the metabolite concentrations (nmoles mg^{-1} of individual dry mass), a linear discriminant analysis (LDA; MASS package; Venables & Ripley, 2002) was conducted on eight classes of samples: three RH classes, each with three exposure durations, with the exception of RH 30%, which had two exposure durations (T_4 and T_8). For LDA analysis, redundancy was avoided by using only molecules sharing less than 80% correlation. *Merizodus soledadinus* adults sampled at T_0 were removed from the discriminant analysis; the physiological status of these fed individuals masked the temporal pattern observed in the three experimental groups. Statistical significance of discrimination was confirmed using a Monte-Carlo permutation test ($P < 0.001$; 10 000 permutations). Statistical analyses were performed on log-transformed data to improve the adequacy of the models' residuals to fit a normal distribution, which was checked using QQ plots and Shapiro-Wilk tests for multivariate normality. Analyses were conducted in R™ 2.11.0 statistical software (R Development Core Team, 2016).

3 - Results

a) *Effects of starvation and desiccation on survival durations*

Strong resistance to starvation was observed in adult *M. soledadinus* (Fig. 1), with the maximal survival duration being 105 d. Death rates were similar between male and female (SURVREG, Z-value = -0.941, $P = 0.347$), and survival data were thus pooled. LT_{50} and LT_{90} reached 51.7 ± 6.2 d and 85.3 ± 9.4 d, respectively.

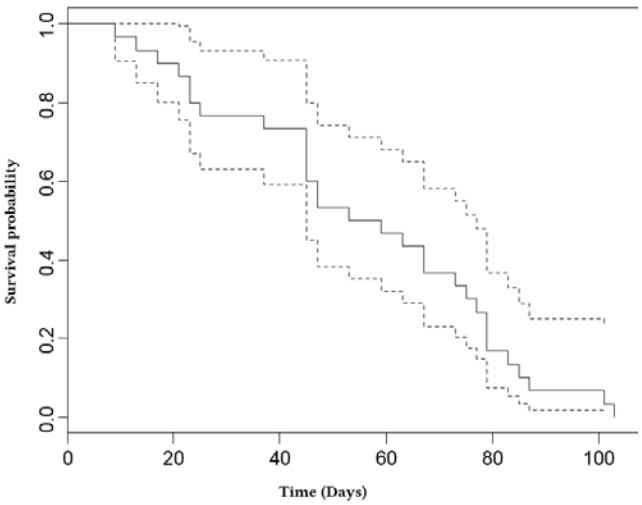
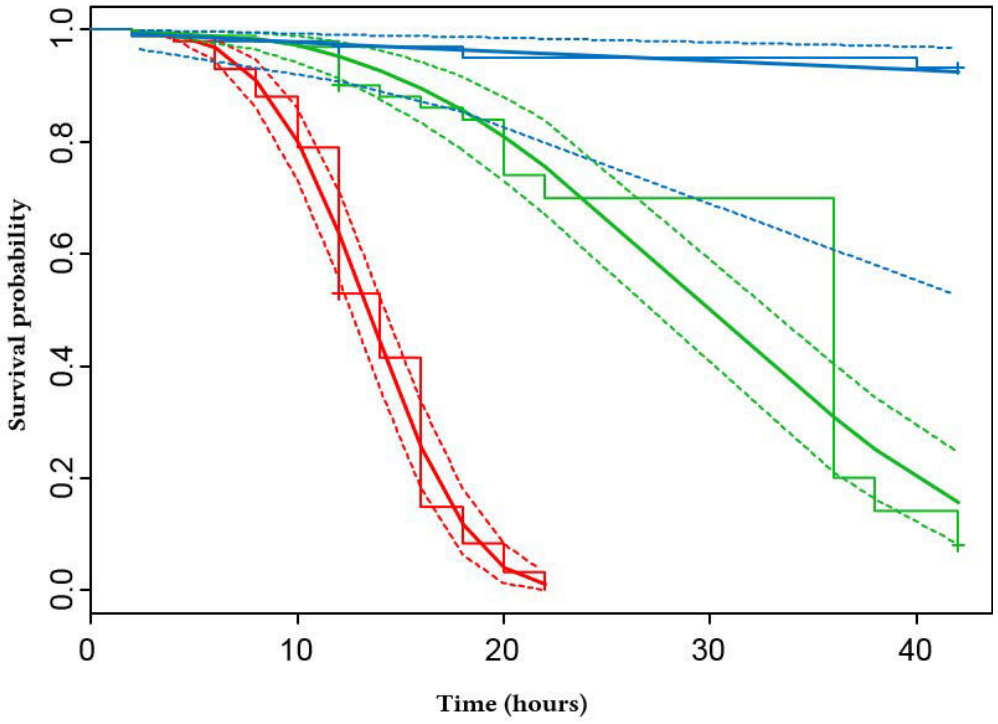


Fig. 1 Survival duration (d) of starved adult *M. soledadinus*. Insects (N = 30) were maintained at 8 °C and supplied with water *ad libitum*. Dotted lines show 95% confidence intervals

Survival curves of adult *M. soledadinus* exposed to 100%, 70% and 30% RH conditions for two days are shown in Figure 2. Significant differences in temporal survival patterns were observed among the three desiccating conditions (SURVFIT, Z-value = 19.2, $P < 0.001$); the highest survival rate among them was observed for the 100% RH group, wherein only 4% (2/50) of the insects died after 48 h. Consequently, it was not possible to calculate LT_{50} and LT_{90} for this treatment condition. Therefore, mortality rate was calculated on the additional sample of 50 individuals maintained under these conditions until death (LT_{50} = 576.96 ± 45.6 h and LT_{90} = 1022.64 ± 71.28 h). Survival of individuals exposed to 30% RH was the lowest of the three conditions (LT_{50} = 13.03 ± 0.48 h and LT_{90} = 19.08 ± 0.75 h) and insects maintained at 70% RH exhibited intermediate survival durations (LT_{50} = 30.37 ± 1.39 h and LT_{90} = 44.44 ± 2.09 h).

Fig. 2 Survival probabilities of adult *M. soledadinus* exposed to 100% (blue), 70% (green), and 30% (red) RH (N = 50 insects per experimental condition). The experiment was terminated after 44 h when most individuals maintained at 70% and 30% RH suffered mortality. Staircases represent mortality data; predicted probability of survival is drawn with bold lines, with 95% confidence intervals (dotted lines)



Adult *M. soledadinus* insects exhibited a non-linear negative response to VPD (R^2 = 0.98831, Fig. 3). Our survival data were consistent with values reported by Todd and Block (1997) at a similar VPD (~ 1.17 kPa). The power regression model fit the measured data well ($P < 0.001$) and demonstrated that slight changes in temperature or RH conditions at a VPD ~ 0.4 kPa had a marked influence on *M. soledadinus* survival duration. Finally, temperature, RH, and VPD conditions at adult *M. soledadinus* field sample sites during the summer period are provided in Table S2. Most of the VPD values were in the range of 0.1-0.5 kPa, but VPD peaked once at 0.93 kPa.

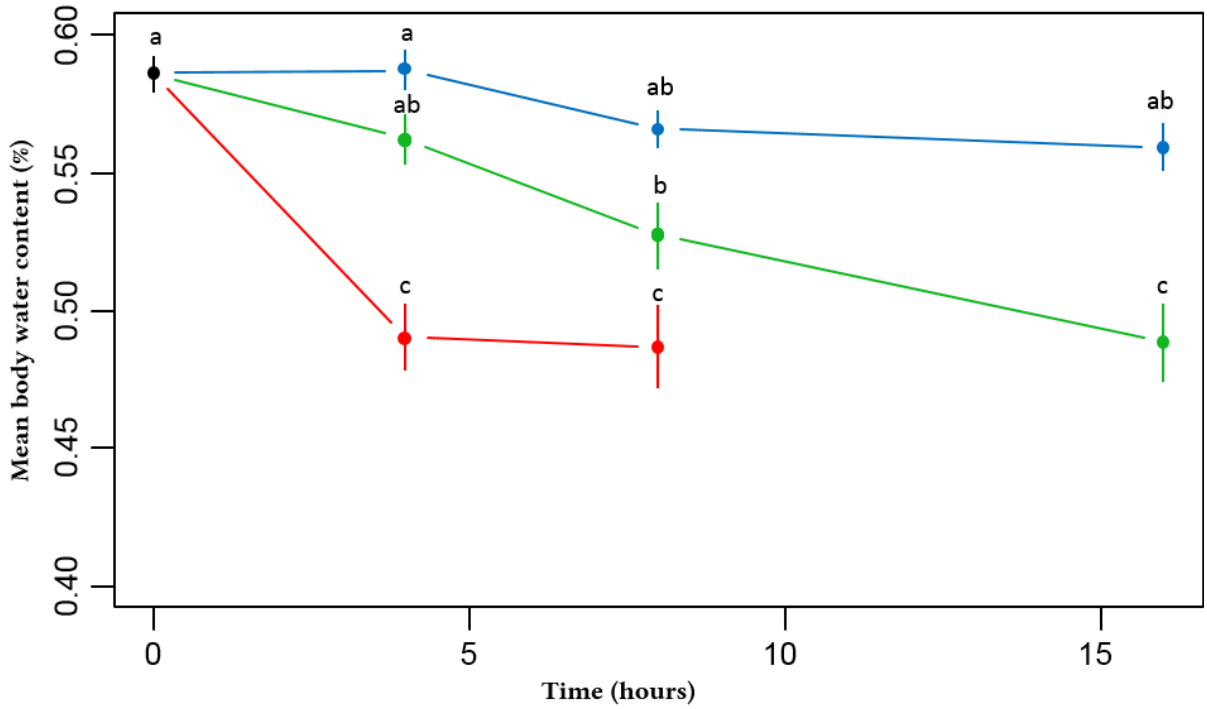


Fig. 3 Variation in body water content (percentage of fresh mass) of adult *M. soledadinus* treated with 100% (blue), 70% (green), and 30% (red) RH. Control *M. soledadinus* (black dot) characterise initial hydration status of the adults (T_0). Distinct letters (a, b, c) indicate differences among experimental treatments (RH and experimental treatment duration). Each dot corresponds to six replicates of two pooled individuals

b) *Changes in body water content due to RH*

As shown in Figure 4, body water content differed in response to RH condition (ANOVA, F_{44} = 33.77, $P < 0.001$). There were main effects of RH (30%, 70%, or 100%; $P < 0.001$) and duration of exposure (0 h, 4 h, 8 h, or 16 h; $P < 0.001$) on body water content, and a RH × duration of exposure interaction ($P < 0.01$) (Table 1). Dry mass also contributed to differences in body water content among individuals ($P < 0.001$) (Table 1); larger adult *M. soledadinus* were characterised by higher body water content.

Tab. 1 ANOVA results computed on body water content of individual *M. soledadinus* exposed at 100%, 70% or 30% RH for 4, 8, or 16 hours (N = 6 replicates of two pooled *M. soledadinus* adults for each experimental treatment)

	Sum Sq	Df	F value	Pr (>F)	*P*
Duration	0.015554	3	15.9919	3.57E-07	0.001
RH condition	0.057566	2	88.7805	3.59E-16	0.001
Dry mass	0.014533	1	44.8259	3.20E-08	0.001
Duration x RH condition	0.006026	3	6.1955	0.001323	0.01

Beetles kept in the 100% RH condition elicited no significant variation in body water content throughout the experiment, whereas beetles kept in 30% RH showed a rapid decrease in body water after 4 h. The 70% RH treatment yielded intermediate results, the beetles' water content levels being similar to that of control beetles after 4 h of 70% RH exposure, and then decreasing to approximate those of 30% RH treated beetles after 16 h (Fig. 4).

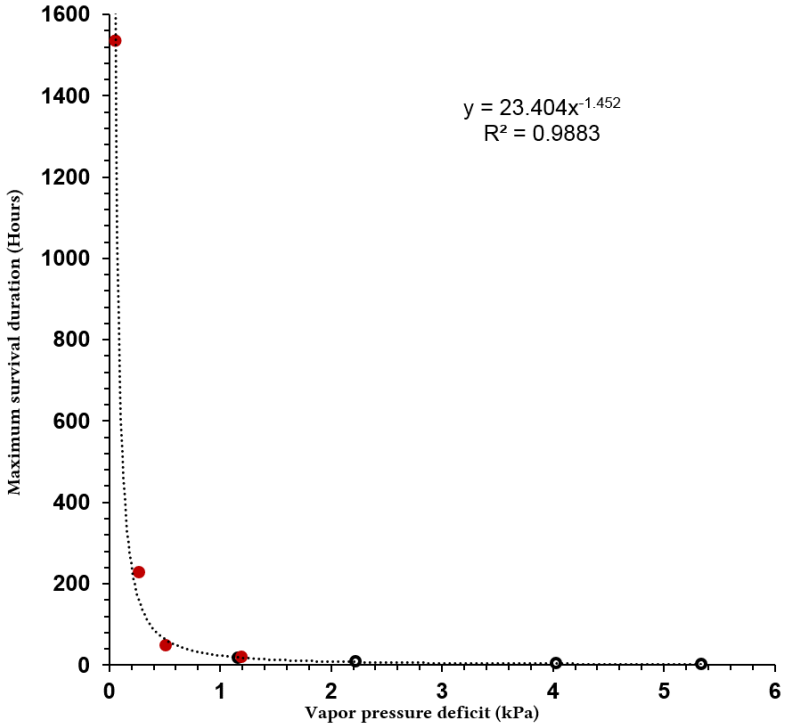


Fig. 4 Maximum survival duration of adult *M. soledadinus* as a function of vapour pressure deficit (VPD). Data from samples originating from Îles Kerguelen (red dots) and South Georgia (data from Todd & Block 1997, dark open circles; Table S1) were combined

c) *Metabolic fingerprinting*

The experimental groups could be discriminated based on their metabolite concentrations (Linear Discriminant Analysis (LDA), $P < 0.001$, Fig. 5). The first LDA axis (LD_1) accounted for 20.5% of the total inertia, and the between-class inertia was 8.24 times higher than the within-class inertia. The variation in several metabolites was associated with LD_1, including sugars (galactose, glucose, maltose) and carboxylic acids (fumarate, malate, succinate) (see Fig. S2 for metabolite variations). Levels were highest in the RH_{100} T_4 group and showed a tendency to decrease as desiccation stress

(RH condition × duration of exposure) progressed. On this axis, trehalose plotted independently from all other metabolites, with the highest levels being observed under increased stress intensity (RH_{30} T_4 and T_8, RH_{70} T_{16}). The metabolites that contributed the most robustly to the LDA second axis (LD_2) were galacturonate, lactate, and mannitol (see Fig. S2 for metabolite variations). On the second axis, RH_{30} T_8 group was plotted to the left, lower quadrant and RH_{70} T_{16} and RH_{30} T_4 groups, which overlapped, was plotted to the right, lower quadrant.

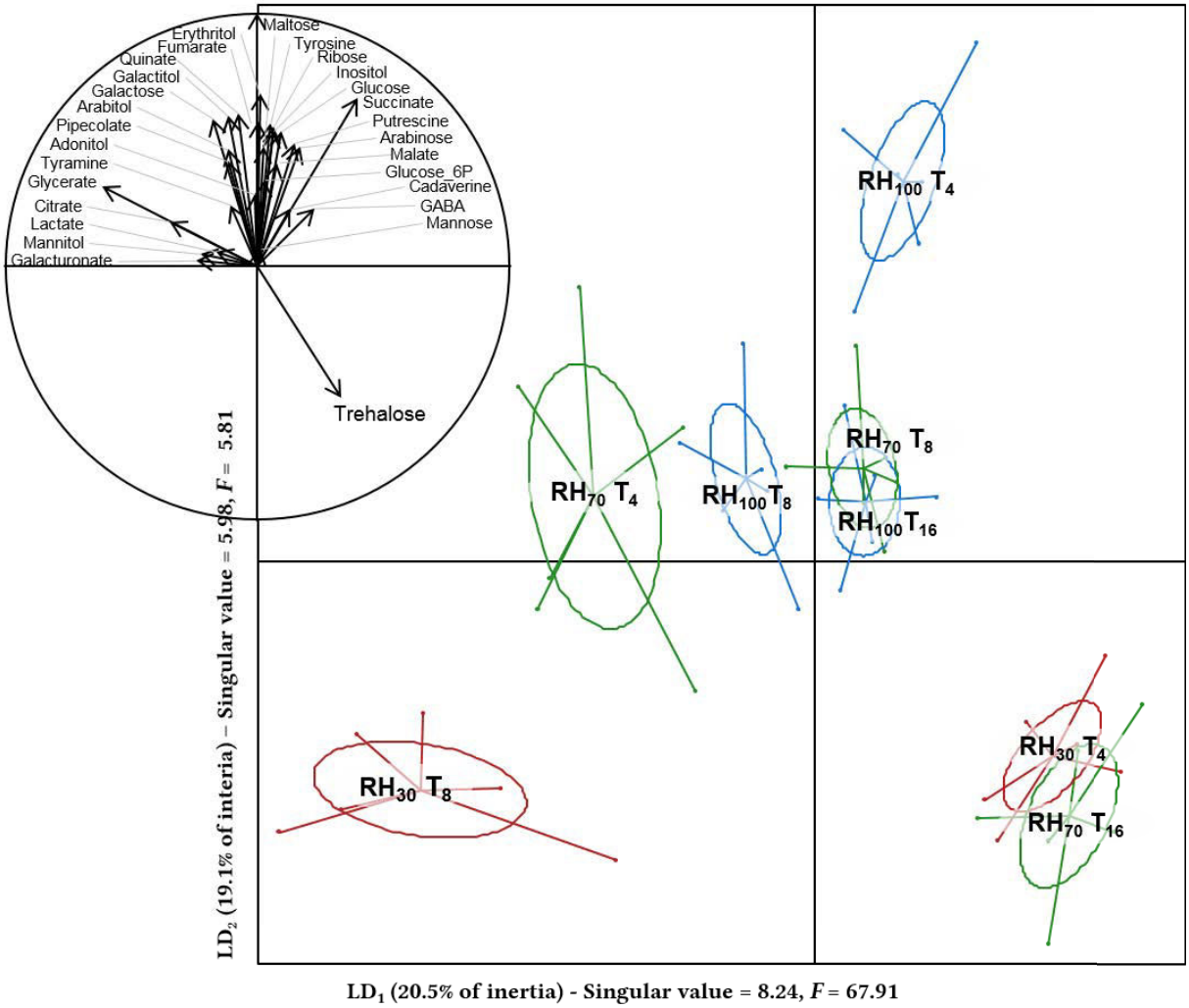


Fig. 5 Projection of experimental groups onto the first LDA discriminant plane. Lines link the samples to the centroid of their class ($N = 6$ replicates for each experimental group). The correlation circle (left) depicts the normed relation (from -1 to 1) between each compound and linear discriminant axes (LD1, LD2). The singular values are the ratio of between-class and within-class inertias. Insects were under three distinct RH conditions: 100% [RH_{100}], 70% [RH_{70}], or 30% [RH_{30}] for three time durations: 4 h, 8 h, and 16 h, except for insects maintained at 30% RH, which were only exposed for 4 h and 8 h (RH_{30} T_4, RH_{30} T_8)

4 - Discussion

Merizodus soledadinus has shown substantial dispersal over the Kerguelen archipelago since the species was introduced in 1913. *Merizodus soledadinus* populations are densely distributed in habitats close to the seashore (Fig. S1) and absent in fell-fields, where water availability and potential invertebrate prey are generally scarcer. It was suggested that this distribution may have been driven

by the availability of potential prey consumed by this predatory carabid beetle (Chevrier, 1996; Laparie, 2011). Large marine vertebrate colonies settle on the shore for reproduction and moulting, which results in large organic matter accumulation in the form of faeces and marine vertebrate carrion. Combined with decaying seaweed, this matter represents a tremendous source of organic input, in which invertebrate fauna, composed primarily of saprophagous taxa, thrives (Vernon et al., 1998). Geographic mapping of *M. soledadinus* distribution at Îles Kerguelen also displayed large populations along watercourses, and negligible to zero adult density in rock fields, peat bogs, and fell-fields, for example (see Renault et al., 2015 for complements). In the present study, our objective was to ascertain the importance of starvation and desiccation in the distribution and dispersal paths of this invasive insect species.

Survival durations in the range of 7-55 d have been reported for starved carabid beetles with body sizes similar to that of *M. soledadinus* (Petersen, 1999; Stone et al., 2001). Our results showed the generalist predator *M. soledadinus* outcompetes these similarly-sized insects, with the maximum survival-to-food deprivation period reaching 100 d. However, the survival capacity of *M. soledadinus* was diminished rapidly in the moderate (70%) and low RH conditions (30%). Todd and Block (1997) reported similar findings from South Georgia specimens, whose mean survival duration at 5% RH ranged from ~8 h to 17 h at 20 °C and 10 °C, respectively. This limited capacity to tolerate desiccation is common in many terrestrial species of austral geographic origins, and is most notable in sub-Antarctic and Antarctic species (Diptera: Hayward et al., 2007; Coleoptera: Todd and Block, 1997), but can also be observed in taxonomically related species from temperate areas (*i.e.* European riparian Carabidae: Andersen, 1985). For polar soil arthropods, such as springtails or insect larva, RH drops can be stressful as soon as 98% RH is reached (Everatt et al., 2014; Hayward et al., 2007); however some oribatid mites can survive for extended periods in 5% RH (Worland and Block, 1986). Adults of *M. soledadinus* are relatively larger than springtails or dipteran larva and therefore have a more favourable surface area: volume ratio. The species possesses a thicker and harder cuticle than springtails, which in addition, do not have a waxy layer on their cuticle surfaces. These character traits might explain *M. soledadinus*' increased capacity to tolerate slightly reduced RH conditions (survival was almost 100% following 2 d at 96% RH and 15 °C).

Temperature and RH value conversions to a single parameter (*i.e.* VPD) revealed similar mortality patterns at comparable VPDs among adult *M. soledadinus* of different geographic origins. Todd and Block (1997) exposed adult *M. soledadinus* to harsh humidity conditions, with all VPD values higher than 1 kPa. We designed our experimental treatments within the range of ecologically relevant RH conditions. Indeed, mean air RH 10 cm above ground level fluctuates minimally and is maintained at ~75% during the austral summer in the Falkland Islands (Bokhorst et al., 2008); at 20 cm above ground level in South Georgia, RH is ~ 50% RH (Ottesen, 1990). The high summer temperatures of 13 °C in the Falkland Islands, and 9 °C in South Georgia result in VPD values of 0.449 kPa and 0.574 kPa,

respectively (Table S1). Environmental data for RH at Îles Kerguelen during the summer months indicated that adult *M. soledadinus* were frequently exposed to VPD conditions ranging from 0.1 to 0.5 kPa under stones, where individuals often thrive (Table S2). Throughout the year, mean monthly atmospheric RH conditions and temperatures ranged from 40% to 60% and +2 °C to +8 °C, respectively, therefore estimated VPD varied from 0.30 kPa to 0.64 kPa (Table S1). A coastal sampling site at Île Guillou (Îles Kerguelen) showed lower RH values (~30%, Source: Prog. IPEV 136, Lebouvier M., Renault D.) 5 cm below ground. The absence of stones at ground level and sunny exposure at this monitored site might explain this observation just below ground level. Daily thermal and RH variations can create water vapour condensation in the morning, making dew accessible to insects. Cumulatively, our data suggested that maximum survival should not exceed a couple of days during the summertime if adult *M. soledadinus* have no access to drinkable water or prey. We hypothesise that *M. soledadinus* beetles will engage in increased predation to compensate for an insufficiency of accessible drinking water, as observed in other predatory insects (Mo & Liu, 2006; Walzer et al., 2007; Hodek & Honêk, 2013).

Our survivorship, body water content, and metabolomics results suggested similar stress levels in insects maintained at 70% RH for 16 h and insects kept in 30% RH for 4 h. These two 'duration x vapour pressure deficit' combinations appeared to be the maximum osmotic stress that *M. soledadinus* could tolerate at micro-temporal scales without lethal injury to individuals. In addition, the rapid decrease in body water content measured at 70% and 30% RH suggested that adult *M. soledadinus* were not able to extract atmospheric water *via* gas exchange (an adaptation very common in xeric species, see Lagadec et al., 1998). Similar conclusions were drawn for Norway riparian *Bembidion* beetles; individuals exposed to desiccating conditions were unable to draw water from atmospheric water vapour and dehydrated individuals only regained fresh mass by drinking and/or eating (Andersen, 1985). Finally, further investigations should consider recovery of dehydrated *M. soledadinus* by resupply with different water intake sources (prey, water droplets).

In the present study, temporal dynamics in *M. soledadinus* body water content was consistent with results reported by Hidalgo et al. (2013), which observed drop in body water content in adults subjected to saline-induced osmotic stress. However, notable differences in survival durations appeared between our work and the one from Hidalgo et al. (2013). Hidalgo et al. (2013) showed that food-deprived *M. soledadinus* adults survived 19.5 d at 4 °C and 70‰ (LT_{50}). Despite the osmotic stress resulting from saline exposure, the high survival duration measured by Hidalgo et al. (2013) likely resulted from access to drinkable interstitial water. Intake of this water, even with increased saline content, might have facilitated tolerance to osmotic stress by increasing hemolymph osmolality or salt excretion. The desiccating stress that we applied to *M. soledadinus* revealed the inability of this insect species to withstand short periods of moderate drought, and emphasised a strong water-dependency. Our results showed rapid death of individuals, suggesting an accelerated onset of dehydration-induced

physiological shutdown of vital metabolic cycles and functions (Wolfe & Bryant, 1999; Kranner & Birtić, 2005; Benoit, 2010).

The set of primary metabolic compounds that we quantified included intermediary metabolites of the tricarboxylic cycle (*e.g.* citrate, malate, succinate), metabolites involved in the mobilisation of body reserves through glycolysis (glycolytic sugars), and several polyols and other sugars circulating in the hemolymph (galactitol, mannitol, erythritol, galactose, ribose). This range of metabolites was demonstrated to be highly responsive in insects exposed to diverse environmental perturbations (Malmendal et al., 2006; Colinet et al., 2012; Hidalgo et al., 2014; Renault et al., 2016). The temporal pattern and group separation observed in the LDA was very similar to the pattern observed for body water content. Food-deprived insects exposed to 100% RH conditions exhibited the highest quantities of most metabolic compounds, and compound levels declined progressively, with significant differences emerging within 4 h, congruent with other studies (Laparie et al., 2012; Laparie & Renault, 2016). This pattern reflects reduction in metabolic activity that spares body reserves (Rion and Kawecki, 2007), resembling entry into a sit and wait strategy (Hervant and Renault, 2002).

Desiccating conditions altered adult *M. soledadinus* survival capacity quickly. Vital physiological (metabolic) functions collapsed progressively in the 30% and 70% RH conditions, suggesting that aerobic ATP-producing pathways could not meet energetic demands at the organismal level, thus triggering the onset of compensatory anaerobic ATP production. Augmented alanine and lactate levels were observed after 8 h into the 30% and 70% RH treatments, and these metabolites do not accumulate under control conditions (alanine amounts declined at 100% RH). These compounds are well-known anaerobic metabolism end products in insects (Hoback & Stanley, 2001). The presence of alanine and lactate likely point to an increased energetic demand (or shortage) of energy production in adult *M. soledadinus*. Glucose levels remained consistently high and nearly constant after 4 h and 8 h of exposure at 30% and 70% RH, and glucose-6-P, fructose-6-P, and threonine concentrations peaked at T_8. Feala et al. (2007) suggested that increased amounts of threonine and glycolytic sugars in *Drosophila melanogaster* exposed to hypoxic conditions may signal a loss of metabolic homeostasis. In fact, most *M. soledadinus* metabolites measured in our study exhibited elevated levels at 30% and 70% RH after 4 h and 8 h of treatment, which later decreased following 16 h under 70% RH conditions. Even if several compounds, particularly amino acids and polyols, accumulate in insects facing desiccation stress, none of these compounds appear to be effective osmoprotectants (Yancey, 2005). Therefore, this pattern may indicate early signs of metabolic dysregulation and, ultimately, the progressive physiological breakdown of dehydrating individuals.

Most amino acids that are directly associated with the tricarboxylic cycle (*i.e.* asparagine, glutamate, isoleucine, phenylalanine, threonine, and valine) had augmented concentrations in *M. soledadinus* following 8 h of 30% and 70% RH treatment conditions. These findings suggest that fluxes

of these amino acids towards the tricarboxylic cycle intermediates were altered, consistent with the generally accepted hypothesis that mitochondrial function is altered in stressed insects (Teets & Denlinger, 2013). Accelerated body water loss in adult *M. soledadinus* measured at 30% RH might have damaged biomolecules, including membranes and proteins, in turn altering profoundly biochemical reactions and metabolic functions (Potts, 1994), resulting in elevated protein breakdown. Our survival and metabolomic data support the notion that there is a rapid attenuation of physiological functions in adult *M. soledadinus* exposed to 30% RH for 4h.

Finally, an interesting pattern emerged in regards to trehalose. This disaccharide is known for its capacity to prevent deleterious effects of dehydration; it is biosynthesised from glycogen and serves to protect membranes against desiccation injuries (Elbein et al., 2003). Among all compounds measured, trehalose showed increased concentrations in beetles exposed to 30% and 70% RH conditions for 4 h and 16 h, which paralleled concomitant decreases in glucose levels. Carbohydrate osmolytes like trehalose might have been accumulated in *M. soledadinus* adults to counteract desiccation-induced stress.

5 - Conclusion

The present study provided novel insights into the biological requirements of the invasive predatory beetle *M. soledadinus* at Îles Kerguelen. Our results indicate that this species has a very high tolerance to prolonged starvation. We also confirmed the crucial importance of water availability for adult survival, which is diminished rapidly when RH conditions fall to 70%. Our findings were consistent with a notable presence of this species in moist microhabitats (littoral areas, sheltered watercourses), and protected habitats with slightly elevated RH (*e.g.* under stones or large wood debris) (Todd & Block, 1997; Renault et al., 2015). During range expansion, the significant starvation resistance we detected for *M. soledadinus* suggests that this species can disperse successfully along pathways where trophic resources are scare, but drinkable water sources are accessible. In localities without water, adults must rely on trophic resources to gain water, and would be expected to exhibit augmented predation rates. In conclusion, our findings indicated that the geographic distribution of *M. soledadinus* populations is highly dependent on habitat RH and water accessibility (taken up directly or through prey consumption). The results of this study provide valuable information regarding dispersal pathway predictions employed by *M. soledadinus* and potential future microhabitats suitable for *M. soledadinus* colonisation.

Acknowledgments

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Port-aux-Français in 2013-2014, are warmly thanked. Finally, we would like to thank two anonymous referees for their helpful comments on a previous version of our manuscript.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jinsphys.2016.08.006

Chapter 5

Effects of elevation on the physiology of the invasive carabid beetle *Merizodus soledadinus* at the Kerguelen Islands

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In prep.

Highlights

- *M. soledadinus* extends its geographic range toward altitudinal habitats, where individuals are exposed to lower thermal regimes and decreased trophic resources, both of which affecting insect's physiology
- Wild individuals were sampled along two altitudinal gradients and along a flat transect (orthogonal to seashore, accounting for decreased trophic resources availability) and we measured morphometric traits, body reserve compounds and free-circulating metabolites contents
- Individuals from altitudinal transects have smaller body size, increased amounts of triglycerides and increased amounts of compounds usually represented in cold-exposed insects (above 200m asl only); females sampled farther from the sea have decreased amounts of protein
- Altitudinal habitats are constraining to adult *M. soledadinus*, probably because of the lower temperature. Ongoing climate change at the Kerguelen Islands may favour further range expansion of this species

1 - Introduction

The geographic distribution and abundance of ectothermic species like insects is highly dependent on climatic conditions. In the context of biological invasions, climatic barrier can represent a significant physiological filter hampering invasion success (Williamson & Fitter, 1996; Walther et al., 2002; Charnov & Gillooly, 2003). Ongoing rising air temperature may favour human-assisted long-distance jumps of exotic insects and subsequent establishment in the release geographic area (Robinet & Roques, 2010). Altitudinal and latitudinal range shift responses to climate changes have been documented for several taxa (Chown & Klok, 2003; Hickling et al., 2006). This is particularly true for insects transported from warmer to cooler regions. In fact, survival and capability to thrive into the released geographic region can be significantly affected by climate change (Hellmann et al., 2008). Rapidly changing climatic conditions in polar regions therefore increase the opportunities for exotic insects to successfully establish (Frenot et al., 2005) and become invasive (Lebouvier et al., 2011). In these regions, climatic change has greatly reshaped climate envelops at different geographic scales, from micro- to large-scale (Smith & Steenkamp, 1990; Frenot et al., 1997; Ingimarsdóttir et al., 2013), opening new opportunities for the establishment of newcomers, or increasing the invasion power of already established species. Changing climatic conditions can also assist exotic alien establishment indirectly, by generating changes in community assemblages elevating the chances of niche availability (Ward & Masters, 2007). There are now several examples that clearly demonstrate that exotic species can benefit from climate-induced disturbances at the community level (Stachowicz et al., 2002; Frenot et al., 2005; Suttle et al., 2007; Vilà et al., 2011).

The subantarctic Kerguelen archipelago undergoes significant climate warming and reduced precipitations over the past few decades. During the period 1951 – 2008, an increase of 1.7 °C of mean air temperature has been recorded, and precipitations drastically decreased since the 1990's (Lebouvier et al., 2011). These climatic changes have long been suspected as important environmental parameters assisting the invasion success of several exotic insects. Among these, the predaceous carabid beetle *Merizodus soledadinus* is the only invasive insect originating from the southern cold temperate area; this insect is native from Patagonia and Falkland Islands (Jeannel, 1962; Roux & Voisin, 1982). The austral origin of the carabid insect raises the question of the physiological thermal limits of this insects and its capabilities to withstand lower or higher climatic conditions of other subantarctic archipelagos where its accidental transportation could be facilitated from the Kerguelen Islands (namely Crozet, Amsterdam & Saint Paul). Altitudinal transects represent a convenient manner to address this assumption, as a fall of 0.5 - 1.0 °C usually occurs every 100 m of increase in altitude at the subantarctic islands (Jenkin, 1975).

As its biogeographical native region suggests, this wingless predator is pre-adapted to the cold oceanic climate encountered at the Kerguelen Islands, and previous studies highlighted a strong tolerance to increased temperatures (Laparie & Renault, 2016). Monitoring records reveal an

accelerated range expansion from the introduction site (Lebouvier et al., 2011), and a distribution along coasts and inland rivers (Ouisse et al., 2016), probably because trophic resources (terrestrial invertebrates) are abundant nearby marine vertebrates colonies, and decreased in inland habitats. Monitoring records of geographical distribution and characterization of habitats colonized by *M. soledadinus* showed recent altitudinal expansion (Renault et al., 2015). These authors highlighted that *M. soledadinus* can now be found up to 400m above sea level (asl), despite a priori unfavourable conditions in altitudinal habitats. Indeed, elevation quickly leads to scarcer availability of trophic resources and temperature decrease (Machac et al., 2011; Hoiss et al., 2012). Studying and comparing highland and lowland populations of *M. soledadinus* would allow to understand biotic and abiotic conditions required for colonisation process, and could allow gaining insights into the effects of climate change, and particularly raising temperature, on the invasive success of this ground beetle.

This study aims to investigate the effect of higher elevation on body size and metabolism of adult *M. soledadinus*. Adult *M. soledadinus* were sampled nearby Molloy and St Malo, where the ground beetles could easily be found up to 250 m above the sea level, the limit after which densities were too low to sample. A flat transect (straight line transect orthogonal to the sea shore) was conducted nearby the research station, to take into account the reduction of the number and diversity of trophic resources, and their possible impact on body size and stores. This flat transect thus allows to distinguish the distribution of trophic resources from the altitudinal effect. Morphological traits were measured, as well as total concentrations of three general reserve compounds in insects (proteins, triglycerides and glycogen) *via* colorimetric assays. Metabolic fingerprints, through Gas Chromatography – Mass Spectrometry analysis, were compared between each transect and elevation. We hypothesise that the reduction of the temperature along elevation gradients should result in a reduction of (i) the body size of individuals, (ii) the amount of body reserves and (iii) the metabolic phenotypes.

2 - Material and Methods

a) Insect sampling along environmental gradients

Adult *M. soledadinus* were hand-collected under stones in March 2013 at the Kerguelen Islands at three distinct localities: Molloy, St Malo and Anse des Papous (see Fig. 1 and supplementary Tab S1). At Molloy and St Malo, two altitudinal transects were realised and started from the foreshore (*ca.* 5 m asl) up to 250m asl; in both locality, sampling sites were stratified at 50 m elevational intervals, starting from 5 m (thereafter refer to as 0) and up to 250 m asl (N = 6 sampling sites). At Anse des Papous, a straight line transect was achieved as a function of the distance to the sea. This transect encompassed eight sampling points (0, 50, 100, 150, 200, 250, 300 and 400 m inland). At each sampling point, GPS coordinates were recorded, and a total of 86 adult *M. soledadinus* were collected: 50 insects were sampled for morphometrics and colorimetric assays (total protein and glycogen), 18 insects (six replicates of three pooled beetles) were collected for triglyceride assays, and 18 insects (six replicates

of three pooled beetles) were collected for Gas Chromatography/Mass Spectrometry (GC-MS) analyses. After collection, insects were directly plunged into microtubes containing 1 mL of 96° ethanol and stored at -20 °C before being further analysed. The physiological status of the specimens may vary from one day to the next, depending on temperature, precipitations or trophic status of the insect. For GC-MS and triglyceride analyses, each replicate was composed of three individuals caught in the morning only on two consecutive days to average environmentally-induced noise and limit possible effects of circadian rhythm on the physiology of the insects. Finally, all samples were done during the same period, and we paid particular attention at the meteorological conditions to avoid having climatic differences among the sampling days.

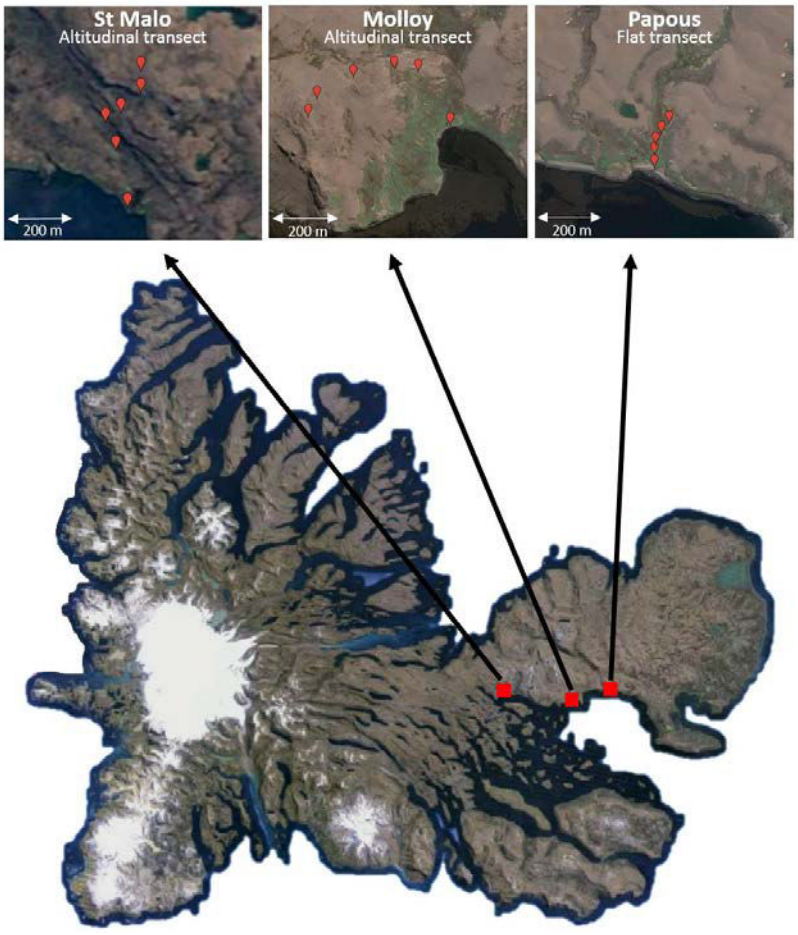

Fig.1 Geographical localisation of the three sampling transects at the scale of the Kerguelen archipelago, and localisation of the sampling sites on each transect

b) Morphological measurements

For each sampling site, 25 individuals were randomly selected (except for specimens sampled at St Malo at 0, 50 and 250 m for which only 12, 21 and 6 beetles were collected, respectively), and further sexed under stereo microscope. Sex ratio was biased toward males in 75% of our samples, and it was necessary to include additional female individuals for Anse des Papous transect (0, 100, 150, 250 and 300 m sampling sites). Pictures of each insect were taken with a video camera (AxioCam ERc 5s, ZEISS, Germany) connected to a stereo microscope. Inter-ocular distance, width and length of the thorax,

length of the right elytra and width of the abdomen (see Fig. 2) were measured by vectorial layouts with AxioVison software. Samples were subsequently vacuum-dried for 15h (Speed Vac Concentrator, MiVac, Genevac Ltd., Ipswich, England), before being individually weighted (Balance XP2U Mettler Toledo, Columbus, OH, d=0.1µg).

Fig. 2 Description of the morphological traits measured on adult *M. soledadinus* (*N*=485). (1) interocular width, (2) pronotum width, (3) pronotum length, (4) right elytra length, (5) abdomen width

c) *Protein, glycogen and triglyceride contents*

Body reserves composition was determined from the six insect sampling sites (0, 50, 100, 150, 200 and 250 m asl) from Molloy and St Malo (altitudinal transects). For Anse des Papous (straight line transect), only five out of the eight sampling sites were analysed, namely 0, 100, 200, 300 and 400m).

Protein and glycogen assays were performed following the protocol developed by Foray et al. (2012) which allows to measure both total protein and glycogen contents from the same sample. These analyses were conducted on six male and six female dried samples; to have comparable samples, individuals whose dry mass was included in between the 25th and 75th percentile of the samples' dry mass distribution were processed. Samples were homogenised in 180µL of phosphate buffer for 90s at 25Hz using a bead-beating device (Retsch™ MM301, Retsch GbmH, Haan, Germany). Following low-spin centrifugation (180 *g*, 4 °C), 10 µL of the supernatant was used to measure total protein content using Bradford's technique. Absorbance of the samples was read at 595 nm. The quantity of protein was calculated from a bovine serum albumin (CAS number: 9048-46-8) calibration curve. The remaining 170 µL were mixed with 20 µL of a solution of Na_2SO_4 at 20% and 1200 µL of methanol-chloroform [ratio 2:1, volume:volume]. Samples were centrifuged (180g, 4 °C) to precipitate glycogen into the pellet. The glycogen concentration was measured through a reaction with 70% anthrone (CAS number: 90-44-8), and the absorbance was read at 625 nm. Glycogen concentration in the samples was calculated based on a glucose standard calibration curve.

For the triglyceride assays, samples consisted of three hand-collected individuals. Six replicates were processed for the six sampling sites of Molloy and St Malo (except for altitudes 0 and 150m at St Malo, for which only five replicates were available), and for five out of the eight sampling sites of Anse des Papous (0, 100, 200, 300 and 400m). We used the analytical procedure described in (Laparie et al., 2012). Briefly, samples were vacuum-dried and weighted before being homogenised in 1050 µL of methanol-chloroform [ratio 1:2, volume:volume] for 90s and 25Hz using a bead-beating device. After centrifugation, 600 µL of the lower phase (chloroform + lipids) were transferred to clean microtubes and dried out overnight under a fume cupboard. The lipidic droplet was redissolved into

200 µL of Triton-BSA buffer and incubated 10 min at 60 °C just before the assays. Manufacturer's instructions were followed for the triglyceride colorimetric assays (Triglycerides, kit reference CC02200, LTA srl, Italy).

d) GC-MS analyses: sample preparation and derivatisation

Concentrations of non-structural carbohydrates, polyols, amino and organic acids were measured using a Gas Chromatography/Mass Spectrometry (GC-MS) platform as described in Khodayari et al. (2013). Analyses were done on pools of three hand-collected carabid beetles (for each insect sampling site, N = 6 replicates for Molloy and St Malo, and N = 5 replicates for Anse des Papous) to depict the metabolic phenotypes of adults from these 17 sampling sites. Dried insect samples were re-dissolved in 600 µL of methanol-chloroform (ratio 2:1, volume:volume) and homogenised using a bead-beating device (Retsch™ MM301, Retsch GbmH, Haan, Germany) at 25Hz for 90s. Four hundred microliters of ultrapure water were added to the samples that were further centrifuged for 10 min at 4000 g at 4° C. Then, 90 µL of the supernatant which contains polar metabolites were transferred to new microtubes, and these aliquots were vacuum-dried (Speed Vac Concentrator, MiVac, Genevac Ltd., Ipswich, England). Dried samples were re-suspended in 30 µL of 20 mg.mL-1 methoxyamine-hydrochloride (CAS Number: 593-56-6, Sigma-Aldrich, St Louis, MO, USA) in pyridine prior to incubation under orbital shaking at 40 °C for 60 min. Following incubation, 30 µL of N-methyl-bis(trifluoroacetamine) (BSTFA, CAS Number: 685-27-8) was added and derivatisation was conducted at 40 °C for 60 min under agitation. Then, 1 µL of each sample was injected using the split mode (25:1). We used the same GC-MS procedure and settings as in Khodayari et al. (2013). Calibration curve samples for 62 pure reference compounds at 1, 2, 5, 10, 20, 50, 100, 200, 500, 750, 1000 and 1500 µM concentrations were run.

e) Statistics

The samples collected at the foreshore level will be further referred to as 'initial sampling point'. For each transect, the Euclidian distance between the initial sampling point and each other sampling point was calculated from the georeferenced coordinates. For both altitudinal transects, Molloy and St Malo, altitude and distance in between the initial sampling point and other sampling points were highly correlated ($r = 0.94$, $P < 0.001$). At Anse des Papous, altitude did not vary, so only the parameter 'distance' was kept as explanatory variable in our statistical analyses. The location of each transect (Papous, Molloy and St Malo) was also added to distinguish site effects.

We examined the effects of sex, transect and distance to the initial sampling point on the five morphological traits measured in adult *M. soledadinus*. As a general measure of body size, we used the coordinates of each individual on the first axis of a Principal Component Analysis (*FactoMineR* package, Lê et al., 2008), which counted for 67.4% of the total variance.

Linear mixed-effects models (*nlme* package, Pinheiro et al., 2007) were used to assess the effect of transect, distance to the initial sampling point and sex on our measured variables, with a random effect of sampling points. According to AICc results (the models are presented in Tab. 1), the most significant model for each variable (body size, protein, glycogen and triglyceride amounts) was retained among six proposed models.

Tab. 1 Set of explanatory variables included for model selection procedure (AICc)

	1	2	3	4	5	6
Distance from the origin point	X	X	X			
Transect	X	X				
Sex	X		X			
Distance x Location				X		X
Distance x Sex					X	X

A total of 46 metabolic compounds were identified *via* the GC-MS procedure. Concentrations were homogenised (nmol.mg^{-1} of individual dry mass) and four molecules (cadaverine, ornithine, quinate and ribose) showing overly high to low concentrations in various samples were removed from subsequent analyses. First, we tested the effects of transect, distance to the initial sampling point, and their interactions on individual metabolite concentrations. MANOVAs were performed to test for potential differences among experimental groups. Second, total metabolite amount of each chemical family was summed for each experimental condition (Free Amino Acids, FAA, N = 13 metabolites; Intermediate Acidic Metabolites, IAM, N = 10 metabolites; Polyols, N = 8 metabolites; Sugars, N = 5 metabolites and Amines N = 4 metabolites). Only two molecules were not included inside families: gluconolactone and glycerate involved in the pentose phosphate pathway and the glycolysis, respectively. Linear Models were then used to compare the calculated amounts for each metabolic family according to the transect and the distance to the origin point. Third, linear discriminant analyses (*MASS* package, Venables & Ripley, 2002) were run on metabolites sharing less than 80% correlation (N = 27 metabolites). Statistical significance of discrimination was confirmed using Monte-Carlo permutation test (P < 0.001; 10'000 permutations). In a first LDA, we plotted the metabolic phenotypes of adult *M. soledadinus* from the three transects. This LDA revealed that our dataset of 27 metabolic compounds was better discriminated transect by transect, with the total variance explained by the first axis at 52.1% (the between-group inertia being 10.7 times higher than the within-group inertia), and by the second axis at 47.8%. LDA analyses were then performed separately. Statistical analyses were performed on log-transformed data to improve adequacy of models' residuals to normal distribution, which was checked using QQ plots and Shapiro-Wilk tests for multivariate normality. All analyses were realised with R™ 3.3.0 statistical software (R Core Team, 2016).

3 - Results

a) Morphological changes along the transects

The statistical model with the lowest AICc value for explaining variations in body size among experimental groups included the parameters 'distance to the initial sampling point', 'transect' and 'sex'. Graphical results are presented in the figure 3**.** Males of *M. soledadinus* are characterised by smaller body sizes than females (χ^2=108.25, P < 0.001). The body size is significantly different depending on the transect (χ^2=7.76, P= 0.021), with individuals from St Malo being smaller than individuals from Anse des papous (z=-2.75, P=0.016). The distance to the initial sampling point had no significant effect on body size (χ^2= 1.72, P = 0.19).

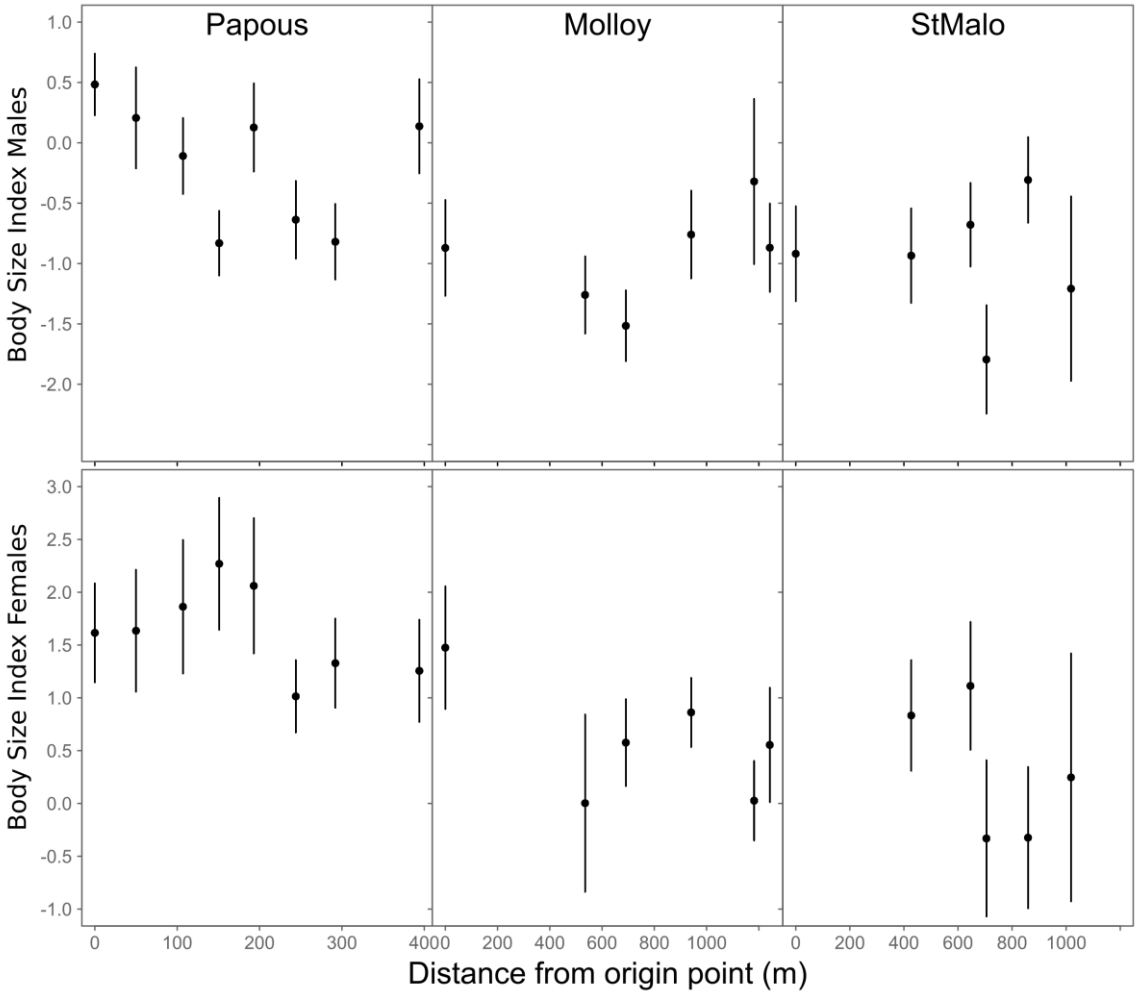

Fig. 3 Variations in body size index (mean ±SE) measured in male and female *M. soledadinus* as a function of the distance to the initial sampling point in the three transects (Papou: straight line transect, Molloy and St Malo: altitudinal transects).

b) Variations in protein, glycogen and triglyceride contents

The model with the lowest AICc value for glycogen concentrations included the parameters 'transect', 'distance to the initial sampling point' and 'sex'. None of these factors significantly impact the glycogen content in samples (transect: χ^2=5.02, P=0.081; sex: χ^2=2.77, P=0.096, distance : χ^2=3.55, P=0.06), even though individuals sampled farther from the sea show a tendency of increased glycogen content (Fig 4).

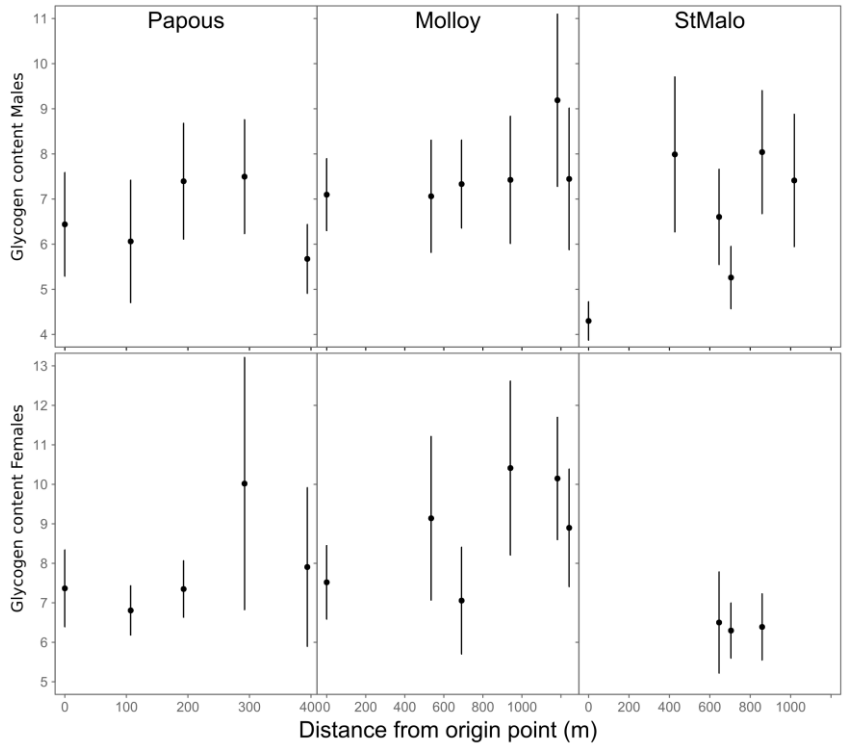

Fig. 4 Variations in glycogen content (in $\mu g.mg^{-1}$ of dry mass; mean ±SE) measured in male and female *M. soledadinus* as a function of the distance to the initial sampling point in the three transects (Papou: straight line transect, Molloy and St Malo: altitudinal transects)

Concerning protein contents, the model with the lowest AICc value included the parameter 'transect' and the interaction 'distance to the initial sampling point x sex'. Females have significantly higher amounts of protein than males (χ^2 = 236.61, P < 0.001, see Fig. 5). As shown in figure 6, the protein content in females decreased with increased distance to the initial sampling point (χ^2 = 17.87, P < 0.001). There is no significant variation of protein content depending on the transect (χ^2 = 0.046, P = 0.97).

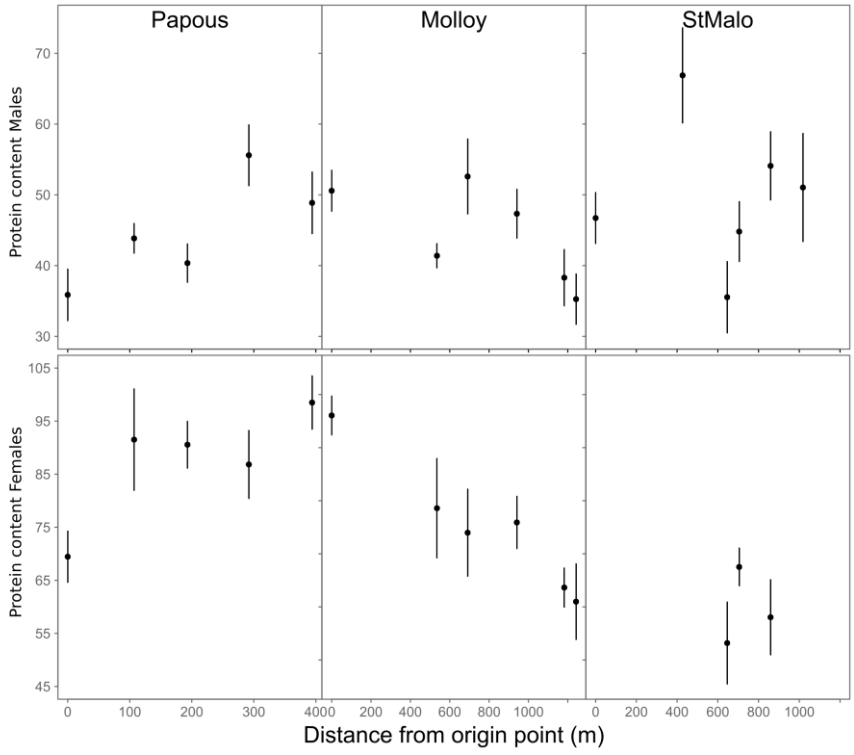

Fig. 5 Variations in protein content (in $\mu g.mg^{-1}$ of dry mass; mean ±SE) measured in male and female *M. soledadinus* as a function of the distance to the initial sampling point in the three transects (Papou: straight line transect, Molloy and St Malo: altitudinal transects)

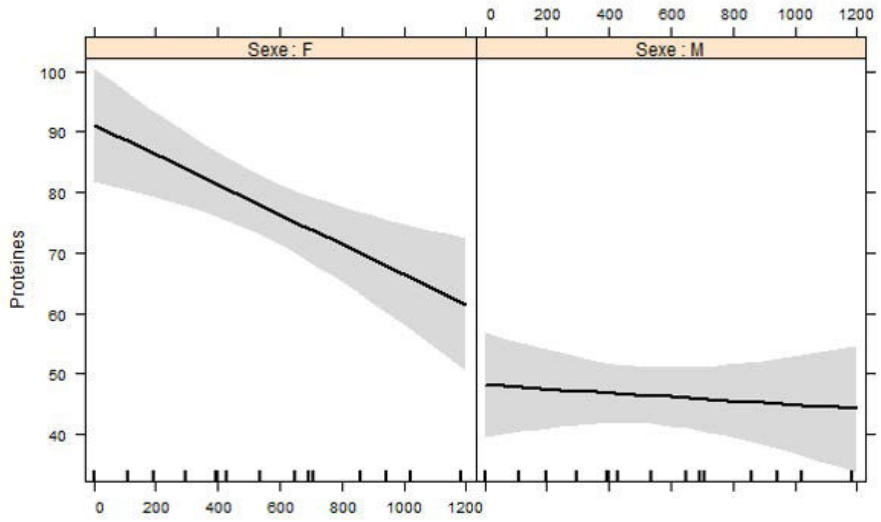

Fig. 6 Interaction plot distinguishing the effect of sex and distance to the initial point on protein content. The following formula was used to compute the linear regression: protein content ~ Site + Distance * Sex. The grey area represents CI 95%

Finally, for the triglyceride amounts, the model with the lowest AICc value included the parameters 'distance to the initial sampling point' and 'transect'. The distance to the initial sampling origin point co-varied negatively with triglyceride contents (χ^2=12.42, P <0.01), as shown in the Figure 7. The samples collected on the two altitudinal transects had significantly higher amounts of triglycerides than the beetles from the straight line transect (χ^2 = 12.75, P =0.0017).

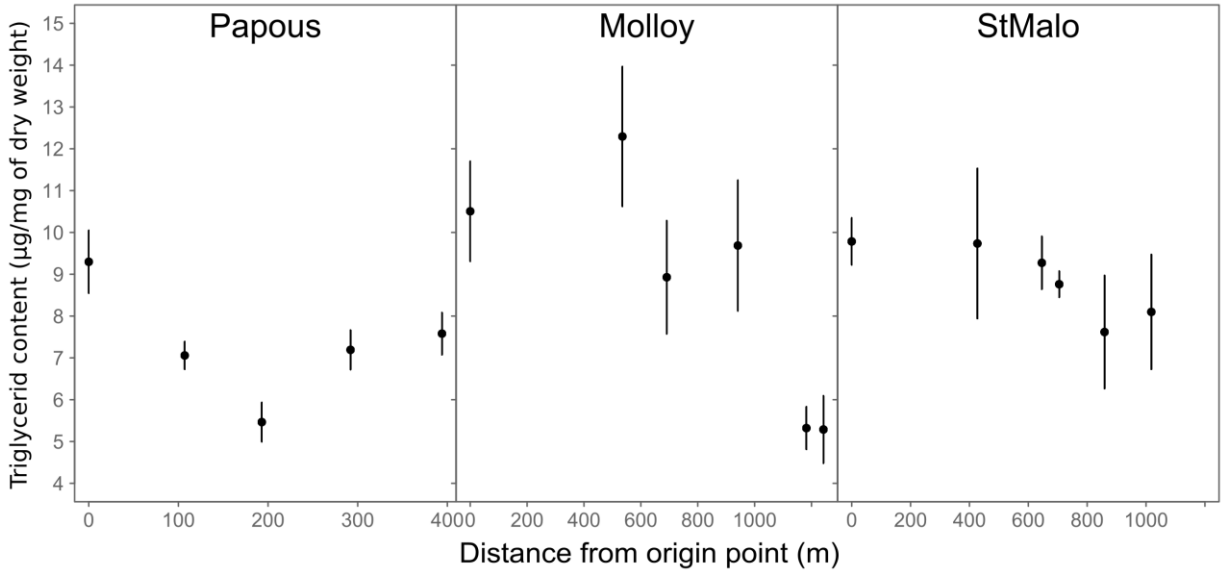

Fig. 7 Variations in triglyceride content (in µg.mg^{-1} of dry mass; mean ±SE) measured in male and female *M. soledadinus* as a function of the distance to the initial sampling point in the three transects (Papou: straight line transect, Molloy and St Malo: altitudinal transects)

c) Metabolic fingerprinting

Results show distinct dynamics of metabolite variation between transects in three out of the five classes of metabolic families (Fig. 8). The individuals sampled along the straight line transect show decreasing amounts of FAA (F = 7.16, P =0.0013), IAM (F = 10.59, P <0.001) and Amines (F = 6.46, P =0.0024) with increasing distance to the initial sampling point, when the amounts in these metabolites

do not significantly vary in the ground beetles sampled along altitudinal transects. Adult *M. soledadinus* sampled along the St Malo transect had lower amounts of polyols compared to the other transects (t =-2.67, P =0.009), and there was no change in the amounts of sugars between transects (F = 1.57, P = 0.21). The distance to the initial sampling point did not impact the amounts of either of the summed metabolic families (FAA: F = 1.07, P = 0.3; IAM: F = 1.6, P = 0.0.21; Polyols: F = 1.06, P = 0.31, Sugars: F =0.24, P = 0.63; Amines: F = 0.23, P = 0.64).

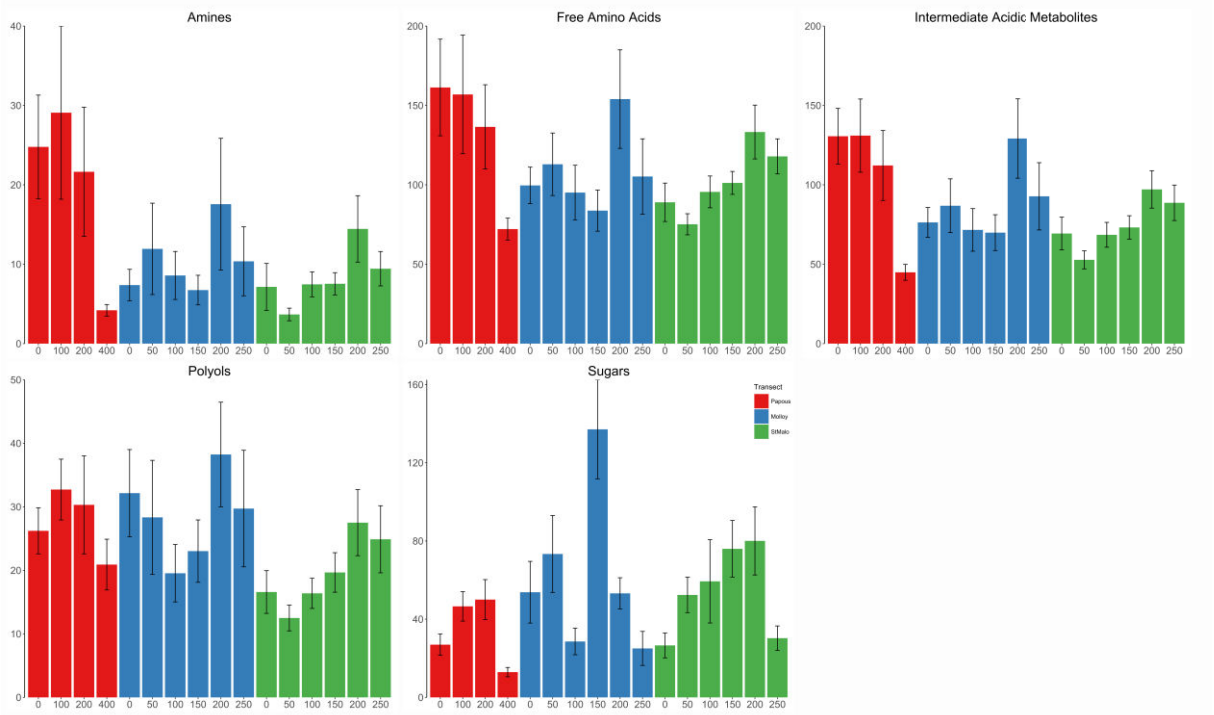

Fig. 8 Content variations in the five metabolite families investigated along the three transects (the straight line transect of Anse des Papou, in red, and the two altitudinal transects of Molloy and St Malo in blue and green, respectively). Measures amounts of metabolites belonging to each family were pooled together and expressed in nmol.mg^{-1} of dry mass (± SE)

The MANOVA performed on the matrix of 42 compounds revealed significant effects of transect (approx$F_{84,94}$ =4.17, P <0.001) and of the distance to the initial sampling point (approx$F_{42,46}$ =2.14, P =0.006). Changes in the concentrations of the 42 detected and quantified metabolites are presented in Fig. 9. This figure encompasses each transect and, either the distance to the initial sampling point (Anse des Papous), or to the sampling altitude (for the samples from Molloy and St Malo). Eighteen compounds showed significantly lower amounts in altitudinal transects as compared with the straight line transect, including eight FAA and six IAM (see supplementary Tab. S2). Four metabolites were found in lower amounts in individuals from St Malo compared to the other transects (namely arabinose, GABA, glycerol 3P and glycine). The distance to the initial sampling point had a significantly positive effect on the amounts of citrate, fumarate, galacturonate and pipecolate.

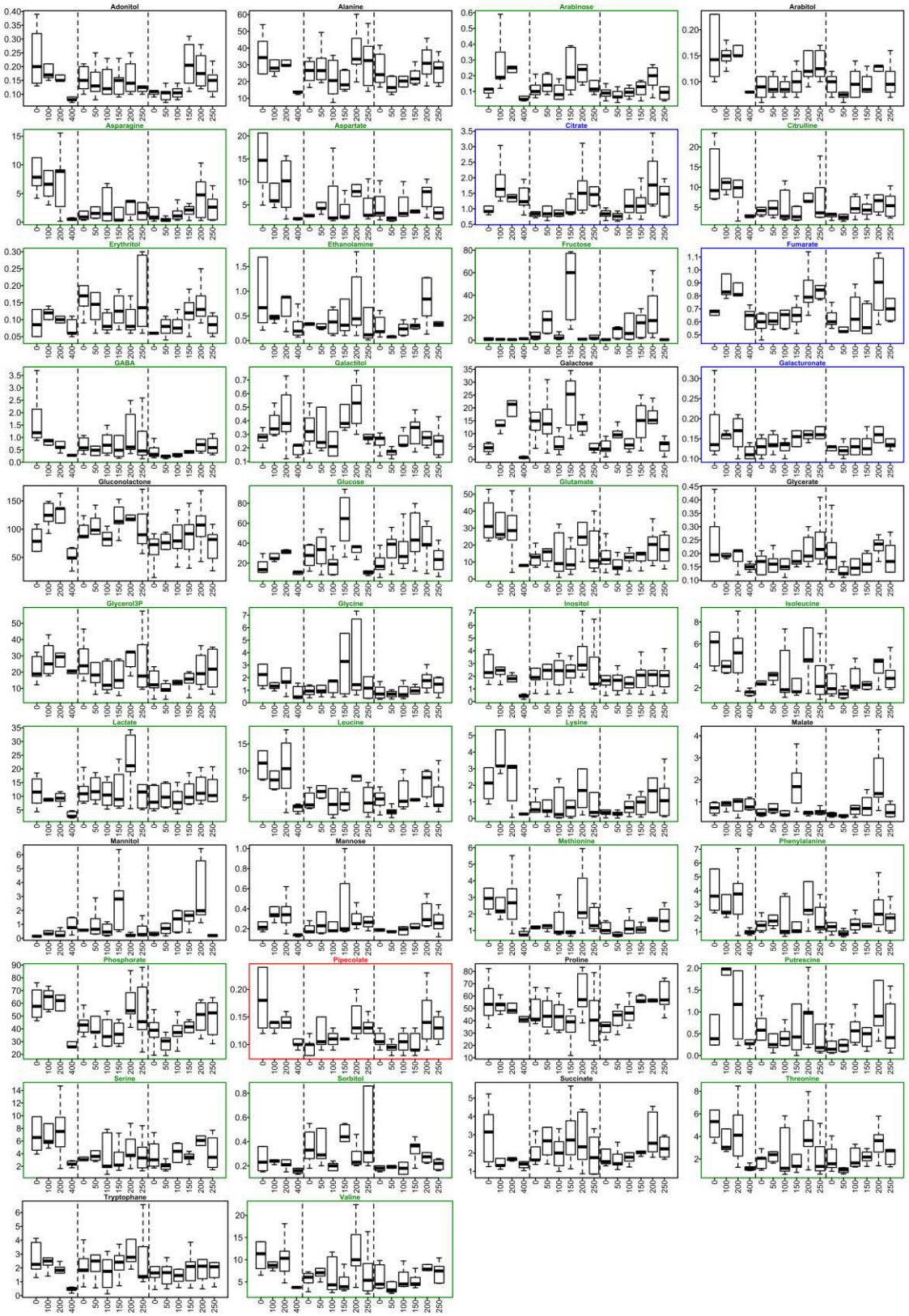

Fig. 9 Variations in concentration (nmol.mg^{-1} of dry mass) observed along the three transects in the 42 metabolites quantified. The three transects are separated by dotted lines, with, from right to left, Anse des Papous, Molloy and St Malo transects. Coloured titles highlight metabolites showing significant variations between transects (in green), according to the distance to the initial sampling point (in blue), or to both (in red). Results of the corresponding statistical tests are outlined in the supplementary Tab. S2

Metabolic variations were further examined for each transect in order to assess if similar discrimination of the sampling groups occurred. For the transect conducted at Anse des Papous, the first axis of the LDA (37.2% of the total variance) separated the samples collected at 0 and 400 m inland from the samples collected at 100 and 200 m inland (Fig. 10.a). This discrimination was partly explained by higher amounts of galactose and glycerol-3-phosphate in the two latter sampled groups. The second axis (34.7% of the total variance) sorted the groups according to the distance to the sea. This axis was mainly supported by pipecolate and succinate whose concentrations increased in groups closest to the sea *versus* higher mannitol concentrations in groups the farthest to the sea.

For the LDA on the Molloy transect (Fig. 10.b), the second axis (24.5% of the total variance) opposed groups sampled at 0 and 100 m asl from those sampled at 150 and 250 m asl, with regularly increasing concentrations of galacturonate and pipecolate. The 150 and 250 m asl sampling points are opposed on the first axis (27% of the total variance), reflecting the peak in concentrations of fructose, glycine or malate at 150m compared with higher elevations. On both axes, the groups sampled at 50m and 200m asl overlapped.

In the second altitudinal transect (St Malo, see Fig. 10.c), the first axis of the LDA (26.2% of the total variance) tended to separate the sampling groups according to the altitudinal gradient. The second axis (22.6% of the total variance) discriminated the 200 m asl sampling group from the others, due to increased amounts of arabinose and mannitol.

4 - Discussion

The carabid beetle *Merizodus soledadinus* is one of the most successful invasive insect at the Kerguelen Islands (Lebouvier et al., 2011; Ouisse et al., 2016). Its geographic distribution has long been restricted to coastal areas (Renault et al., 2015), but since the early 2000's, it is sampled at an increased frequency along altitudinal gradients in this archipelago. In the present study, we speculated that the ongoing colonisation of altitudinal habitats since the past decade has been facilitated by changes in climatic conditions that occurred at the Kerguelen Islands (Lebouvier et al., 2011). The geographic distribution of *M. soledadinus* at the Kerguelen Islands also represents a good opportunity for assessing the effects of microhabitat conditions and thermal gradients on the morphology, biochemistry and physiology of field-sampled specimens.

Body size of insects is expected to increase over altitudinal gradients, as a result of colder thermal conditions. This pattern of body size variation has been conceptualised as the Bergman's and temperature-size rules (Atkinson, 1994). Consistently, Chown and Klock (2003) reported a significant augmentation of the body size of four weevil species sampled along a 1000 m-altitudinal gradient at the subantarctic Marion Island, whose seasonal climatic conditions are similar to those of the Kerguelen Islands. However, we did not observe this allometric relationship in adult *M. soledadinus*, and body sizes of this carabid beetle were even decreased at higher altitudes. In spite of the above

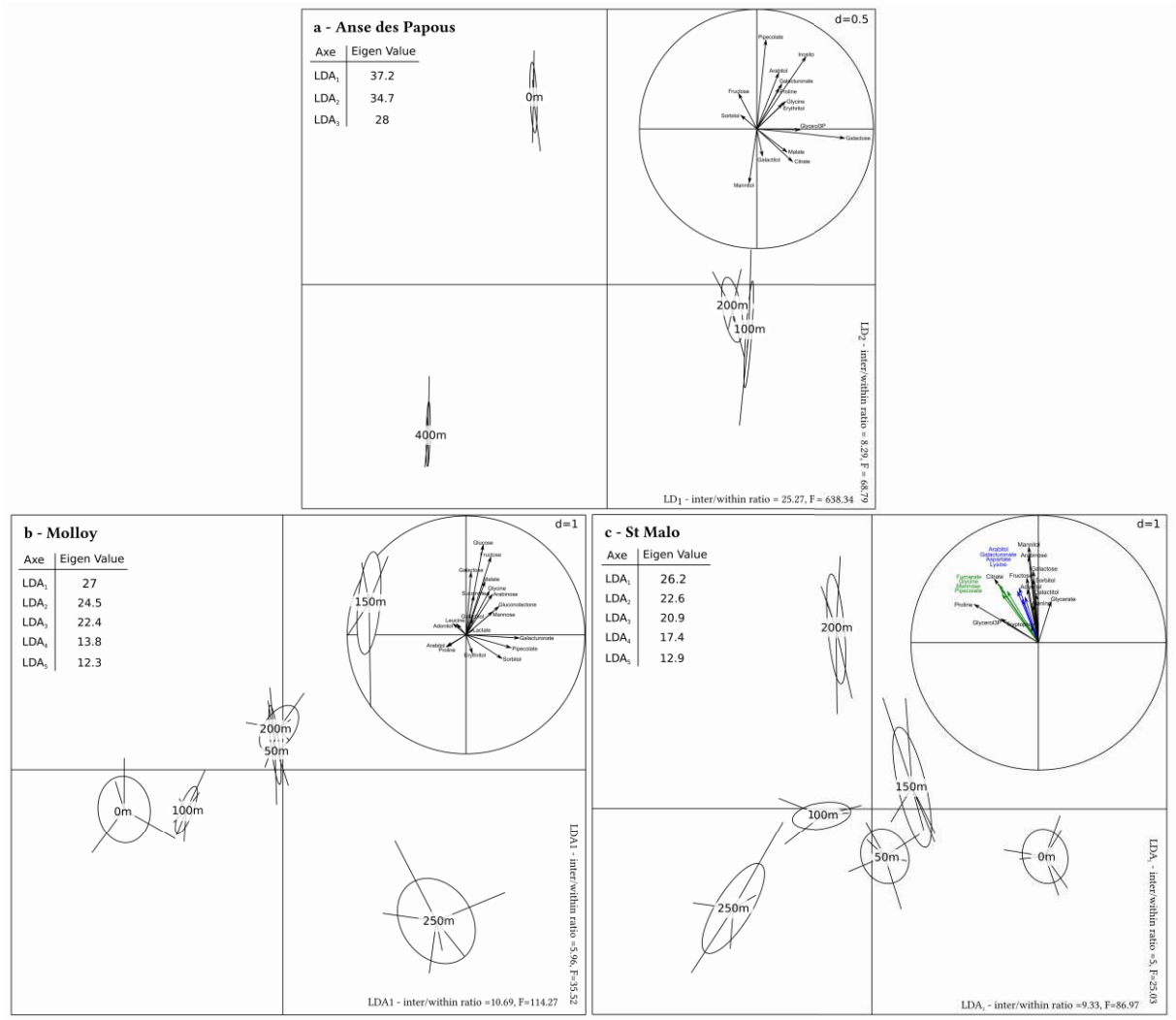

Fig. 10 Projection of the metabolic profiles of adult *M. soledadinus* sampled along each of the three transects onto the first two axes of the LDA. Each sampling site is characterised by its distance to the initial sampling point (for the straight line transect of Anse des Papous – **a**) or by its altitude (Molloy and St Malo altitudinal transects – **b** and **c**). The eigen values represent the proportion of variance explained by each existing axis of each of the analyses. The correlation circle depicts the relation between each metabolites kept in the analyses (compounds sharing less than 80% of correlation) and the discriminant axes. For each axis, the ratio of inter-class and within class inertias, and the associated F-statistics are provided

mentioned rules, the progressive reduction of body size along altitudinal gradients is not rare, and has already been reported in a variety of insects (Krasnov et al., 1996; Ciplak et al., 2008).

For instance, at the subantarctic Heard Island, Chown and Klock (2003) observed that body size of weevils was decreased with altitude, a pattern that was opposed to the one reported from weevils of Marion Island. Thermal and trophic resources are more constraint at Heard Island than at Marion Island, and resource limitation imposed to weevils may have affected the growth efficiency of these sedentary insects (Chown & Klok, 2003). At the Kerguelen Islands, patches of trophic resources become more sporadic as altitude augments, limiting nutrient availability for the voracious adults of *M. soledadinus*. In parallel, the structure and abundance of vegetation shifted along the two altitudinal

transects: starting from grassland dominated by *Poa annua* at sea level, it shifted to continuous cover of *Acaena magellanica* at middle-hillside, which progressively became scarcer and patchy in rocky fell-fields at 250 m asl. Changes in vegetation cover are known to impact the structure and diversity of invertebrate communities (Gremmen et al., 1998), in turn affecting trophic resources qualitatively and quantitatively for both larva and adults of *M. soledadinus*. Yet, a nearly constant body size was measured in carabid beetles from the straight line transect, which was designed to assess the effects of decreased availability of trophic resources as a function of the distance to the sea. As a result, decrease of trophic resources does not necessarily affect body size significantly in adult *M. soledadinus*, and nutrient availability is not the exclusive cause of the smaller body sizes measured in elevated habitats.

Temperature is expected to decrease from 0.50 to 1.0 °C when elevation increases by 100 m at the subantarctic islands (Jenkin 1975), with the rate of thermal decrease being the highest from 0 to 150 m asl (Davies & Melbourne, 1999). This suggests that temperature was decreased by 1.5 to 2.5 °C from the sea level to the highest sampling point in our experimental design. At 5 m asl at the Kerguelen Islands, mean monthly temperatures measured 10 cm below the ground level, where this carabid beetle thrives, vary from ca. 1.2 to 8.4 °C from winter to summer (Lalouette et al., 2012). Mean monthly temperatures are lower than 3.8 °C during six over 12 months, and lower than 2.4 °C during 4 months (Lalouette et al. 2012). These data suggest that *M. soledadinus* sampled above 150m asl spend a significant part of the year at temperatures around 0 - 1 °C. These thermal conditions are highly restricting for this insect whose acute thermal sensitivity has been reported in earlier investigations (Lalouette et al., 2012; Laparie & Renault, 2016). Indeed, metabolic activity and efficiency of adult *M. soledadinus* is highly reduced when temperature falls around 4 °C (Lalouette et al. 2012), and thermal conditions are largely suboptimal for adult *M. soledadinus* when they are dropping to near 0 °C (Lalouette et al. 2012; Laparie & Renault 2016). It is likely that adult *M. soledadinus* from the two altitudinal gradients experience temperature-imposed resource and physiological limitations when altitude increases.

A range of studies reported decreased in development rates in insects of smaller size when energy assimilation is restricted (Partridge & French, 1996; Moczek, 1998; Nijhout, 2003; Mirth & Riddiford, 2007). During development, the rate of growth of insects is partly driven by protein synthesis (van der Have & de Jong, 1996), and this enzymatically-based process should work more slowly at lower temperatures and/or should be tone down when resources are limiting. There was no significant effect of the type of transect on protein and glycogen contents, but body proteins were significantly reduced in females along altitudinal transects, which, in addition, were characterised by a reduced body size. This finding supports the idea of the strong importance of thermal-imposed resource limitations in altitudes. Finally, triglyceride contents were decreased along the three transects, which could depict energetic adjustments according to the nature of preys available. Diet-

related differences have already been measured in adult *M. soledadinus* fed with enchytreids or fly maggots (Laparie et al. 2012); carabid beetles food-deprived for two days before being refed with fly maggots exhibited higher triglyceride amounts.

Environmental metabolomics has been proven a powerful tool allowing to delineate insect-environment interactions (see reviews by Viant, 2008; Bundy et al., 2009). Field-sampled organisms are expected to show high inter-individual variability, which we attempted to smooth by working on pools of three insects. The global inter-site comparison revealed that insects from the three populations (Anse des Papous, Molloy and St Malo) were characterised by distinct metabolic phenotypes, despite a relatively small geographic distance separating the three sampling sites. Interestingly, metabolic phenotypes of the specimens from the two altitudinal gradients (Molloy and St Malo) did not overlap, suggesting that insects experience distinct ecological situations along these two gradients. Indeed, we have no genetic data for these three populations, but genomic analyses revealed that allelic diversity is rather similar in specimens among populations of *M. soledadinus* sampled along the invasion gradient (see Chapter 1).

Several amino acids, including the essential isoleucine, leucine, methionine, phenylalanine, threonine and valine, were characterized by higher amounts in the straight line transect as compared with the two altitudinal gradients. Essential amino acids cannot be synthesized by insects and are only acquired from trophic resources, suggesting higher food intake in adult *M. soledadinus* from the straight line transect. Nutrient limitation in essential amino acids can greatly constrain females' fecundity (O'Brien et al., 2002), as these metabolites contribute for a significant part of egg carbon. The speculated higher food intake of adult *M. soledadinus* from the straight line transect is consistent with the variation of protein concentration in females, which remained constant (or even increased) in this transect, whereas it was reduced in the altitudinal transects. Moreover, amino acids represent a prominent source of nitrogen for insects, and nutrient limitation during larval stage can have significant consequences on the phenotypic traits of the adult, by modifying development duration, in turn affecting body size and fitness of adult (Tigreros, 2013). Several proteins of larval origin are indeed catabolized by female for egg production (Wheeler et al., 2000; O'Brien et al., 2002), but additional latent effects of nutritional limitation can be expected (Tigreros 2013). Yet, even though nutrient limitation appears from our dataset from specimens collected along altitudinal transects, it remains uncertain if this nutrient limitation arose from the reduction of the availability of trophic resources or a byproduct of the progressive temperature decrease along the gradient, progressively affecting metabolic activities.

Metabolic phenotypes measured in specimens from the straight line transect (Anse des Papous) highly discriminated the samples collected at 0 and 100 m (which grouped with 200 m) on the one hand, and those sampled 400 m far from the seashore on the other hand. The insects from the seashore (0 m) exhibited high concentrations of inositol and pipecolate. At this sampling site, carabid

beetles can thrive in the surroundings of sea wrecks where the salinity level can be high (from 35 to 70 parts per thousand). Saline environments impose osmoregulatory constraints to organisms, which can be supported by accumulation of specific osmolytes (Yancey, 2005). The accumulation of inositol and pipecolate were already observed by (Hidalgo et al., 2013) in adult *M. soledadinus* experimentally exposed to increasing salty conditions. Specimens sampled 400 m far from the seashore had lower amounts of amino acids, TCA cycle intermediates and sugars as compared with the three other groups. Even if we have no data for the metabolic rates of these specimens sampled 400 m far from the seashore, this observation might suggest a lowered metabolic activity resulting from nutrient limitation. Three metabolic families were found in lower amounts in altitudinal transects, with no effect of the distance to the initial sampling point (namely free amino acids, intermediate acidic metabolites and amines), this pattern being similar as the one recorded for body size variations, and, similarly to the '400 m' sample from the straight line transect, may reveal reduced metabolic activity.

Even if different metabolites were involved into the discrimination of the groups of the two altitudinal transects, the general picture was rather similar. Specimens sampled along the seashore differed from the other samples, and those sampled at 200 (St Malo only) and 250 m asl were largely opposed. In this latter group, there was a slight tendency of augmentation of the amounts of circulating amino acids, intermediate acidic metabolites and polyols. This pattern has been reported earlier in a large range of cold-exposed insect (Colinet et al., 2006; Lalouette et al., 2007), including in adults of *M. soledadinus* (Laparie & Renault, 2016). Higher amounts of free amino acids represent signs of cold stress in insects, thus adding another evidence of the restrictive thermal conditions for the physiology of adult *M. soledadinus* at 200 - 250 m asl. The discriminant analysis of the two altitudinal transects, St Malo and Molloy, also revealed a similar ranking of the sampling sites, with a gradient of lower elevation toward higher elevation sites along the first axis. It can be suggested that this pattern stems from the same factor acting upon individual's physiology in the two altitudinal transect, which is probably temperature. At intermediate elevations, metabolic phenotypes exhibited important inter-site variations, most probably resulting from the specific micro-environmental characteristics of these habitats. Inter-individual variance (age, sex, reproductive status, nutritional status at the time of the collect) may explain the high within-group variance, as well as potential divergence in origin due to high dispersal abilities of this ground beetle: an individual collected on a transect may not have spent all its life at the same level and may have reached lower of higher elevation after its emergence (larvae have much lower dispersal abilities compared with adults). This phenomena might have blurred some of the results but our findings at higher investigation scales suggest it is not as frequent as to smooth all patterns along transects.

5 - Conclusion

This study highlights the phenotypic changes of adult *M. soledadinus* sampled along terrestrial and altitudinal gradients at the Kerguelen Islands. Body size of adults were reduced as altitude

increased, and we conclude that this pattern most probably results from the suboptimal temperatures encountered during larval stages at altitudinal habitats. Indeed, thermal conditions from 200 m asl onwards progressively impact the overall physiology of this insect. These results suggest that climatic changes have preconditioned the invasion process of *M. soledadinus* in altitudinal habitats, as populations of this species were nearly absent at low altitudes before 2003, while the recorded mean air temperature increase of 1.7 °C between the mid-1960th and 2010 (Lebouvier et al. 2011) have probably permitted the colonisation of altitudinal habitats. If temperature increase continues, this insect will have the possibility to flourish at its currently colonized altitudinal habitats, and could even progress and colonize higher altitudes, provided the availability of trophic resources.

Acknowledgments

We are very grateful to Maxime Dahirel for his statistical support and advices, and to the helpful IPEV logistics team that greatly facilitates field work. This research was granted by InEE-CNRS (Enviromics call, 'ALIENS'; Zone Atelier Antarctique et Subantarctique), and supported by the French Polar Institute (IPEV, program 136) and the CNRS (Zone Atelier de Recherches sur l'Environement Antarctique et Subantarctique). Metabolic analyses were performed on the PLAY platform (UMR CNRS Ecobio, Rennes, France).

Supplementary material

Tab. S1 GPS coordinates of the sampling sites along each of the three transects

	Sampling Site	Latitude	Longitude	Altitude (m)	Distance from origin point (m)
Papous	MR 0	-49.345091	70.170657	0	0
	MR 50	-49.344643	70.170674	0	50
	MR 100	-49.344125	70.170649	0	107
	MR 150	-49.343732	70.170666	0	151
	MR 200	-49.343355	70.170777	0	193
	MR 250	-49.342906	70.171034	0	244
	MR 300	-49.342513	70.171451	0	292
	MR 400	-49.341733	70.172401	0	394
Molloy	RJ 0	-49.340408	69.82201	0	0
	RJ 50	-49.336666	69.820862	50	535
	RJ 100	-49.33484	69.81988	100	691
	RJ 150	-49.334229	69.821391	150	941
	RJ 200	-49.332949	69.823367	200	1182
	RJ 250	-49.331579	69.823382	250	1242
St Malo	KO 0	-49.361968	70.06634	0	0
	KO 50	-49.357872	70.062506	50	427
	KO 100	-49.357642	70.059627	100	646
	KO 150	-49.358327	70.054793	150	705
	KO 200	-49.359952	70.050551	200	859
	KO 250	-49.361342	70.049556	250	1018

Tab. S2 Numerical outputs of the post-hoc test of the MANOVA performed on the 42 quantified metabolites. The multivariate linear regression included distance to the initial sampling point and transect as explanatory variables. The factorial reference for transect was Anse des Papous, the straight line transect. The extent of variation in metabolites compounds from this reference are respectively expressed in the two columns 'Molloy' and 'St Malo'. Significance levels: $P<0.05$ *; $P< 0.01$ **; $P< 0.001$ ***

ref= Papous	Molloy		St Malo		Distance	
	t-value	*P*	*t-value*	*P*	*t-value*	*P*
Adonitol	-1.347	0.182	-1.569	0.12	0.203	0.839
Alanine	-0.743	0.46	-1.526	0.131	0.587	0.559
Arabinose	-1.286	0.20191	-2.913	0.00455 **	1.659	0.1008
Arabitol	-1.047	0.298019	-1.468	0.145749	-0.712	0.478083
Asparagine	-2.878	0.005031 **	-3.543	0.000638 ***	0.127	0.899431
Aspartate	-2.294	0.02419 *	-3.026	0.00326 **	0.036	0.97134
Citrate	-2.545	0.012698 *	-2.392	0.018896 *	3.524	0.000679 ***
Citrulline	-2.476	0.01522 *	-3.24	0.00169 **	0.617	0.53883
Erythritol	1.867	0.0652 .	-0.243	0.8082	-0.167	0.8679
Ethanolamine	-2.083	0.04019 *	-2.973	0.00382 **	0.514	0.60876
Fructose	1.989	0.0498 *	1.402	0.1644	0.214	0.8307
Fumarate	-3.176	0.002069 **	-3.478	0.000791 ***	3.198	0.001931 **
GABA	-1.484	0.1413	-3.142	0.0023 **	0.776	0.44
Galactitol	-0.07	0.944	-1.956	0.0536 .	0.96	0.3399
Galactose	1.284	0.202	-0.141	0.888	-0.155	0.877
Galacturonate	-0.899	0.371	-0.792	0.4304	2.068	0.0416 *
Gluconolactone	-0.22	0.826	-1.588	0.116	1.243	0.217
Glucose	1.275	0.2056	1.683	0.0959 .	0.893	0.3743
Glutamate	-2.9	0.004721 **	-3.711	0.000364 ***	0.766	0.445853
Glycerate	-1.438	0.1541	-1.853	0.0673 .	1.359	0.1778
Glycerol3P	-0.7	0.48527	-2.693	0.00849 **	0.971	0.33444
Glycine	-0.94	0.3518	-2.591	0.0112 *	1.448	0.1511
Inositol	1.306	0.195	-0.304	0.762	0.662	0.509
Isoleucine	-2.26	0.02634 *	-3.122	0.00244 **	0.558	0.57839
Lactate	1.61	0.111	0.38	0.705	1.483	0.142
Leucine	-2.66	0.009376 **	-3.48	0.000781 ***	0.12	0.905035
Lysine	-2.648	0.00960 **	-3.194	0.00196 **	0.397	0.69249
Malate	-0.656	0.513	-0.913	0.364	1.036	0.303
Mannitol	0.577	0.5656	1.34	0.1837	1.014	0.3135
Mannose	-0.563	0.575	-1.633	0.106	1.12	0.266
Methionine	-2.902	0.004690 **	-3.758	0.000309 ***	0.566	0.572828
Phenylalanine	-2.445	0.01652 *	-3.245	0.00167 **	0.323	0.74753
Phosphorate	-2.191	0.03115 *	-3.105	0.00257 **	1.365	0.17592
Pipecolate	-3.328	0.001282 **	-3.717	0.000356 ***	2.252	0.026827 *
Proline	-1.966	0.0525 .	-1.006	0.317	1.847	0.0682 .
Putrescine	-2.317	0.02286 *	-2.723	0.00781 **	0.614	0.54065
Serine	-2.719	0.00790 **	-2.973	0.00381 **	-0.01	0.99204
Sorbitol	0.792	0.43055	-0.745	0.45816	1.254	0.21329
Succinate	0.751	0.455	0.081	0.936	0.346	0.73
Threonine	-2.559	0.01223 *	-3.17	0.00211 **	0.269	0.78844
Tryptophane	0.911	0.365	-0.382	0.703	0.702	0.484
Valine	-2.228	0.02843 *	-3.123	0.00243 **	0.731	0.46653

General Discussion

The fast-growing populations of non-native arthropod species, their ecological niche shifts during invasion process, and their accelerating expanding ranges in the areas of introduction remain under deep investigation. In this thesis manuscript, I have focused on the main determinants assisting the invasion success of the predatory carabid beetle *Merizodus soledadinus* invading the Kerguelen Islands. Among the different factors hampering or assisting its ongoing invasion success, the performance of several biological traits including fecundity, longevity, stress resistance or dispersal in newly colonised regions is pivotal. Thus, several studies were undertaken to complete our knowledge of the factors supporting the range expansion of *M. soledadinus* at the Kerguelen Islands.

1 - Summary of the main results within the framework of this thesis

Chapter 1 – The long-living adults of *M. soledadinus* are year-round active and no egg-laying period exists

The first chapter of this dissertation focus on life history traits of this ground beetle in the introduced area that remained yet unexplored. The adult life span was determined under controlled conditions, the seasonal activity was monitored in natural conditions through regular pitfall trappings, and temporal variations of egg load and body reserves (glycogen, proteins, triglycerides) were analysed in wild individuals over an 18-month period (**Chapter 1**). The maximum adult lifespan reached 710 days, with half of the individuals surviving around 240 days in the experimental setting. Females carry eggs evenly over the year (8.94 ± 3.56 eggs per female). It was not possible to pinpoint any clear egg-laying period from our data, thus questioning the earlier assumed semelparity of this species. Specimens were caught at each trapping session, demonstrating that activity is maintained year round in this predatory insect, but peaks were observed during the austral summer. Year-round trappings demonstrated the continuous activity of *M. soledadinus* over seasons, with a peak during Austral summer that may be associated with higher temperatures. Body stores (glycogen and proteins) did not vary significantly over the year, suggesting that adults feed continuously despite different prey availability between summer and winter, possibly *via* diet shifts. The amount of triglycerides increased at the onset of summer, and may be associated with the higher summer locomotor activity and/or more intense predation and reproduction.

Chapter 2 - Genomic studies reveal the low diversity and the absence of structure of *M. soledadinus*' populations at the Kerguelen Islands

We hypothesised that the introduction of *M. soledadinus* at the Kerguelen Islands would have resulted in a strong reduction of genetic diversity (bottleneck) in the established population compared with counterparts collected at a biogeographic scale covering the subantarctic distribution of this insect. Moreover, we suggested that the range expansion of *M. soledadinus* at the Kerguelen Islands would have resulted in a spatial sorting along the invasion gradient. In order to address these

assumptions, molecular approaches (Restriction Site Associated DNA, RADseq) were undertaken (**Chapter 2**). These datasets constitute the first available genetic information on this ground beetle. A first try-out was performed by constructing a RADseq library including 16 individuals from the Kerguelen Islands. The success of this first library led to the building of two distinct datasets.

First, 31 individuals from Patagonia (N =12), South Georgia (N =9) and Kerguelen Islands (Port Couvreux, N =10) were included into two RADseq libraries designed to reconstruct biogeographical relationships between native specimens from Patagonia and those from the introduced populations of subantarctic archipelagos (Kerguelen Islands and South Georgia). As expected, introduced specimens showed decreased levels of genetic diversity compared with individuals from the native area (Patagonia). Interestingly, a higher level of genetic diversity was observed in adult *M. soledadinus* from South Georgia than from the Kerguelen Islands. This finding suggests that multiple introductions may have occurred in South Georgia (closer to the Falkland Islands, sheep transportations probably occurred between these two archipelagos). Thus, admixture may have contributed to this increased level of genetic diversity. Sequenced imagoes from Port Couvreux (Kerguelen Islands) were characterized by drastic reductions in genetic diversity compared with the other two locations, supporting the single introduction event of this insect at the Kerguelen Islands.

Second, five RADseq libraries including individuals sampled from 10 populations of the main island of the Kerguelen archipelago, and distributed along the invasion gradient, were built. The maximum heterozygosity was found in specimens from Port Couvreux, which, again, supports the hypothesis of a unique historical introduction site. The most recent established population, sampled at Val Studer, is the only population presenting a positive F_{IS} (0.004), and for which the Hardy-Weinberg exact test is significant (P =0.008), showing a deficit in heterozygotes. Our results highlight the absence of genetic structure among the ten sequenced populations, even though heterozygosity values show a decreasing pattern from oldest to youngest populations. This later result supports the long-term monitoring data of the range expansion of this species, *i.e.* colonisation of the mainland was achieved via natural dispersal events from the initial introduction site. Conversely, individuals from the population of Estacade display very homogenous patterns of diversity, which could be explained by human-assisted introduction of few founder individuals from the research station. To complete this study, we need to include individuals from the research station to explore the eventual anthropogenic influence on the current distribution of *M. soledadinus*, and individuals from the Falkland Islands to finalise biogeographical reconstruction of this species.

Chapter 3 – Evidences for phenotypic differentiation between front and core populations suggest spatial sorting dynamics

Laparie et al. (2010, 2013) reported a gradual increase of body size in adult *M. soledadinus* sampled from populations with different their residence times along the invasion gradient. In the second chapter of this dissertation, we prolonged the work of Laparie et al. by analysing the

phenotypic change of adult *M. soledadinus* along the invasion gradient using a vast set of morphological, behavioural and physiological traits (**Chapter 3**). Individuals were sampled from eight populations of the main island of the Kerguelen archipelago, with residence times ranging from 103 to 4 years since the initial establishment. We first confirmed the morphological gradient previously highlighted by Laparie et al. (2013). We then found that individuals sampled from recently settled populations can support longer periods of starvation and exhibit higher locomotor activity than their counterparts sampled from formerly established populations. These findings support the idea of higher dispersal capacities in specimens at the invasion front. Yet, activities of enzymes involved in energetic metabolism are similar among individuals from these different populations, except for PGI. Activity of this later enzyme was increased in individuals from the older population, which contrasts with most of the available literature. Using GC-TOF-MS metabolomics, a clear cut-off appears in the metabolic phenotypes of specimens sampled from older and younger populations, in both sexes. These promising preliminary results require more in-depth analyses of the potential different metabolic pathways favoured in front populations *versus* formerly established populations. No differences were found in the propensity to leave a patch, although more precise inquiries of dispersal behaviour are required. This study, by combining phenotypic characterisation of several traits, provides an integrated approach of the phenotypic changes that occur during the range expansion of this species, and suggest the involvement of spatial sorting in this phenotypic differentiation.

Chapter 4 – Relative humidity and water accessibility have a prominent role in driving invasion path and settlement of *M. soledadinus* populations

A field-based assessment of the habitat distribution of the invasive carabid beetle *M. soledadinus* was conducted at the Kerguelen Islands (Renault et al., 2015). Results emphasised humid habitats as one key element of the insect's realised niche. In a third study, we thus quantitatively evaluated how water availability and trophic resources governed the spatial distribution of this invasive predatory insect on the Kerguelen Islands (**Chapter 4**). Specifically, expected survival time under food and water stress (relative humidity conditions of 100%, 70%, and 30% RH) and changes in a set of primary metabolic compounds induced by such stresses (GC-MS, metabolomics) were determined. We found that adult *M. soledadinus* supplied with water *ad libitum* were highly tolerant to prolonged starvation (LT_{50} = 51.7 ± 6.2 days). However, food-deprived insect survival rapidly decreased at moderate (70% RH, LT_{50} = 30.37 ± 1.39 hours) and low (30% RH, LT_{50} = 13.03 ± 0.48 hours) relative humidity conditions. Consistently, body water content rapidly decreased in insects exposed to 70% and 30% RH. Metabolic variation emphasised the effects of food deprivation in control insects (exposed to 100% RH), in which a progressive decline of most glycolytic sugars and TCA cycle intermediates was observed. Overall results of our study indicated that the geographic distribution of *M. soledadinus* populations is highly dependent on habitat relative humidity and water accessibility (directly available for the insect or through prey consumption).

Chapter 5 – Altitudinal habitats are physiologically constraining for *M. soledadinus*, but still undergo colonisation

Altitudinal habitats are being colonised by *M. soledadinus* since the early 2000's, possibly driven by ongoing climatic changes. In a fifth step, we investigated the effects of altitudinal gradients on the morphology and physiology of adult *M. soledadinus* (**Chapter 5**). Wild imagoes of *M. soledadinus* were sampled along two altitudinal transects, from sea level to 250 asl, and along a 400m-long straight transect. The straight transect was designed in order to consider the effect of decreasing availability of food resources farther from sea shore from the effects of decreased temperature in altitude. Results show decreased body size in individuals sampled along altitudinal transects. We suggest that this pattern could be attributed to the lower thermal conditions experienced by juveniles during developmental period in altitude. Concerning body reserves, glycogen amounts remain similar along the three transects, the protein amounts decreased in females sampled farther from the seashore and increased levels of triglyceride were found in individuals sampled in altitude. Metabolomics analyses allowed to quantify 42 free-circulating metabolites and the resulting metabolic phenotypes distinguish samples from the sea shore from those sampled at 200m asl. This difference probably results from the saline conditions encountered at the sea shore, which requires increased levels of osmoprotective compounds. We concluded that thermal conditions encountered in altitudinal habitats are suboptimal for adult *M. soledadinus*, but ongoing climate seems to assist the colonisation of these habitats, provided that biotic requirements match.

2 - Other experiments conducted during this thesis, but not included in the dissertation

Several other experiments were run during the three years of this PhD, but are note presented into this manuscript. These works can be combined into three main studies.

1/ We used molecular approaches (13 microsatellite markers) to examine kinship structure in adult *M. soledadinus* at a fine geographic scale. Six hundred and thirty nine individuals were sampled in 2010 at the locality of Cataractes, along a 1500m transect parallel to the seashore. Sampling sites were spaced by increasing intervals (0m, 10m, 50m, 100m, 250m, 500m, 1000m and 1500m) At each sampling point, individuals were collected under *c.a.* eight different stones. Kinship reconstructions along this transect will allow to understand the dispersal dynamics of *M. soledadinus* at a small scale (Ouisse, Guiller, Renault, data not presented). This experiment is a remake from a study described in Lalouette (2009) whose 85m transect was insufficient for drawing conclusions. Microsatellites markers are already genotyped, the dataset needs now to be analysed (Coancestry software).

2/ During range expansion, individuals from front populations are predicted to exhibit differences in stress response because of their more frequent exposure to novel environmental conditions (and potentially new stressors). We designed an experiment to explore this assumption, by exposing individuals (10 replicates of 10 beetles per population) sampled from six populations at the

Kerguelen Islands (residence times of 103, 36, 21, 10, 6 and 4 years since local establishment) to stressful conditions. Specifically, we tested the effects of desiccation (similar procedure as described in **Chapter 4**), starvation (similar procedure as described in **Chapter 3**) and fluctuating thermal regimes (from 8 °C to 28 °C with a stepwise increase/decrease of temperature during daytime, [L:D 12:12], and constant 8 °C condition at night). In an additional experimental condition, individuals were exposed to all of three environmental factors simultaneously. While these words are written, this experiment is still running at the research station of Port-aux-Français (Ouisse, Hotte, Renault, data not presented).

3/ Localities recently colonised at the Kerguelen Islands benefit from precise estimation of the arrival (establishment) of populations of *M. soledadinus*. At the locality of Isthme Bas, the arrival of *M. soledadinus* has been anticipated, by setting pitfall traps in four sites in 2005. In two of these locations (IS_1 and IS_2) *M. soledadinus* specimens were already present at the start of the experiment. Conversely, in the two other trapping sites (IS_3 and IS_4) the first individuals arrived sporadically between 2006 and 2010 (see Fig.1). From this dataset, characteristics of the very first colonisers could be explored, including their sex, amounts of body reserve, number of eggs in females, and eventually the age.

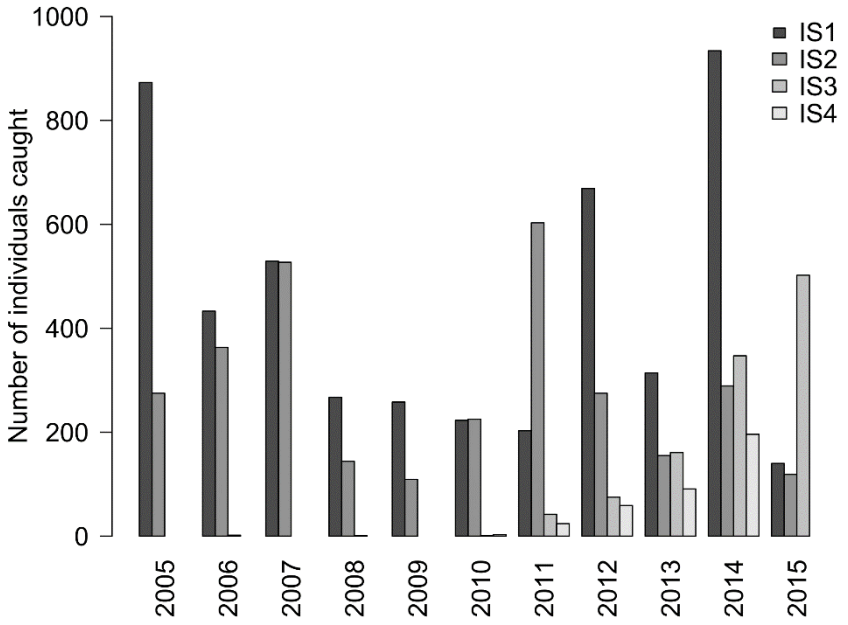

Fig. 1 Number of *M. soledadinus* caught per year in pitfall traps installed in the locality of Isthme Bas from 2005 to 2015

3 - This invasion case presents several advantages...

The invasion process of *M. soledadinus* at the Kerguelen Islands constitutes a valuable opportunity to study eco-evolutionary processes, and their underlying mechanisms, that occur during range expansion. As outlined in the general introduction of this dissertation, combining the characteristics of the Kerguelen Islands together with those of *M. soledadinus* makes this model system unique as compared with the majority of invasion cases. The likely unique introduction of *M. soledadinus* at the

Kerguelen Islands, from a known geographic area, and to a unique location (Port Couvreux) is very rare in the context of biological invasions. In this work, by using genetic analyses, we have been able to corroborate the hypothesis of the single introduction event of this species (**Chapter 2**). Together with the long term monitoring of the terrestrial biodiversity, genetic results confirmed the chronosequence of the geographical expansion of this insect at the Kerguelen Islands.

Evidence accumulates for progressive phenotypic changes along the invasion gradient of *M. soledadinus*, and these changes are likely imputable to spatial sorting (**Chapter 3**). The biological and ecological data collected for *M. soledadinus* (Laparie et al., 2010, 2013; Renault, 2011; Lalouette et al., 2012; Hidalgo et al., 2013; Renault et al., 2015; Laparie & Renault, 2016); **Chapter 1, 4 and 5**) are essential pieces of the puzzle for our understanding of the population dynamics, potential invasion routes and ecological niches of this invasive insect at the Kerguelen Islands. This knowledge could be further used to parametrise range expansion models to predict the future expansion of *M. soledadinus* on the Kerguelen territory, and propose mitigation measures. For example, in the case of the invasion of Australia by the cane toad, authors used ecological information, anthropological elements and climatic conditions of the current distribution of *Rhinella marina* to predict the extent of habitats that could be invaded by this species (Sutherst et al., 1996; Urban et al., 2007).

Considering the data that are now available for *M. soledadinus*, we suggest that the combination of distributional, genetic and ecological data could allow to create a resistance matrix that would indicate the most favourable habitats together with the least cost paths to join them at the Kerguelen Islands. Such a model could help predicting the extent and the expansion rate of *M. soledadinus*, in this archipelago. This model should primarily include the topography of the Kerguelen Islands, as we know that *M. soledadinus* can only be found up to 400m asl at present. Altitudinal habitats are currently physiologically constraining (**Chapter 5**) for this insect, meaning that this kind of habitats would be attributed increasing cost values when elevation increases. Also, this ground beetle has a coastal distribution and low tolerance to desiccation (**Chapter 4**). The model should thus accounts for increased habitat values farther from the sea or major watercourses. Like in other cases of biological invasions (Thomas et al., 2001; Butin et al., 2005; Mau-Crimmins et al., 2006), modelling the distribution of *M. soledadinus* in the invaded areas could help to understand the mechanisms underlying geographic expansion and to predict future directions of the ongoing expansion. From a practical standpoint, this would greatly help stakeholders to undertake localised conservation measures in this nature reserve (Réserve Naturelle des Terres Australes Françaises).

At the Kerguelen Islands, individuals of *M. soledadinus* have no apparent competitors. Nevertheless, a high level of intra-specific competition occurs in microhabitats where population density is very high (more than 400 individuals can be found in less than 10 minutes, pers. obs.). Under controlled conditions, cannibalistic interactions were observed among adults, but also between adult and larvae. Kubisch et al. (2013) highlighted that kin competition can represent a strong force triggering the initiation of dispersal in invasive species. This should be even truer in environments

where patches of favourable habitats are highly fragmented, but characterised by a low environmental stochasticity. Our genetic results (**Chapter 2**) revealed an absence of population structure along the invasion gradient of *M. soledadinus* at the Kerguelen Islands. The reduced number of founder during the introduction event may explain this finding, and have resulted in a high degree of relatedness among individuals. Meanwhile, we were expecting that relatedness would have been increased in recently established populations (at the range edge), as a result of sequential founding events from the core population of Port Couvreux. Coupled to assortative mating among dispersers, these processes should have putatively increased relatedness among individuals at the invasion front, a phenomenon that we did not observe. We suggest to re-assess this finding by conducting kinship reconstructions (Blouin, 2003) in future studies.

In the past century, *M. soledadinus* has been introduced to South Georgia and Kerguelen Islands, two subantarctic islands sharing climatic conditions that are not strongly different than those encountered in the native area of this insect species. These "replicated" invasion situations could allow designing interesting comparative studies in order to search for general ecological and evolutionary patterns accompanying the introduction and geographic expansion of *M. soledadinus* in environments for which they seem to be pre-adapted.

Theoretical predictions developed in Burton et al. (2010) stipulate that expansion dynamics should be reduced if the introduced species faces interspecific competition. This idea could be tested with *M. soledadinus*, as, in South Georgia, this species must compete with another carabid beetle *Trechisibus antarcticus* (Dejean 1831), whose populations densities and geographic distribution are higher (Brandjes et al., 1999; Convey et al., 2011). Such comparative investigations would require ecological inquiries on both species in South Georgia, but could ultimately allow to compare and quantify the effects of intra- and inter-specific competition on range expansion. Overall, this case offers promising research perspectives to understand processes associated with invasion and expansion of species, whether they are invasive or not.

4 - ...but also some limitations

a) *What about the other developmental stages, and more particularly larvae?*

Even if we have provided new knowledge on the autecology of *M. soledadinus* (**Chapter 1**), there remain some gaps in our understanding of the reproductive biology and life cycle of this insect. As earlier pinpointed by Laparie (2011), attempts to rear this species were so far inconclusive, and, in general, the rearing of carabid beetles is not easy. As a result, we are still missing several features regarding the biology of this insect, such as the trophic and abiotic requirements of the larvae, recruitment rates, competition and predation pressures or movement abilities. These missing elements can represent significant issues, because understanding reproductive cycle is fundamental for drawing and understanding population dynamics. The results presented in **Chapter 1** show that females carry a relatively low but constant number of eggs over the year, which somewhat contrasts with the high

abundances of *M. soledadinus* in several localities of the Kerguelen Islands. Controlling reproduction of *M. soledadinus* through rearing would permit to study heritability of phenotypic traits, especially for those traits that vary between core and range populations. These data would supply us with information on selection for dispersal-related traits, as requested in spatial sorting, through QTL mapping for example (Stinchcombe & Hoekstra, 2007).

b) *Inter-individual variability*

Several characteristics of the individuals, such as sex, trophic status and age, can affect physiological measurements (body reserve compounds, metabolic activity), and induce inter-individual variability, especially for field-sampled specimens. Experiments aiming at measuring physiological traits of *M. soledadinus* adults were designed in order to smooth these differences, and limit inter-individual variability among experimental replicates. Sex has been taken into account in almost all experiments. In addition, individuals' physiological status (trophic and thermal conditions) has been standardised by keeping individuals in controlled conditions (and fed *ad libitum*) at least a week prior to being used for the experiments. Inter-individual variability was further reduced in some experiments by measuring physiological traits from pools samples of two or three individuals. Despite this procedure, the exact age of each insect remains an unknown parameter.

In **Chapter 1**, we first demonstrated that the longevity of adult *M. soledadinus* can exceed 14 months, and that there is seemingly no distinct oviposition period. For future investigations, it should be possible to distinguish individuals by classes of age through morphological proxies such as mandibular or claws usury (commonly used on beetles; Butterfield, 1996; Matalin & Makarov, 2011) or through the quantification of cuticular hydrocarbons that vary with age (Trabalon et al., 1992; Caputo et al., 2005; Cook et al., 2006). Both of these possibilities are under current investigations, using young (red cuticle) adult *M. soledadinus* sampled in March 2013 at Port-aux-Français, kept in controlled conditions in parallel to the longevity experiment (**Chapter 1**), and regularly sampled from zero to 16 months after the start of the experiment.

c) *What does* M. soledadinus *eat?*

The trophic regime of *M. soledadinus* has been evaluated by proposing a range of potential preys (Renault D., unpublished results). Meanwhile, these tests were conducted under controlled conditions, to evaluate feeding preferences through choice experiments. A careful examination of the diet of *M. soledadinus* requires metabarcoding investigations of gut content (Krehenwinkel et al., 2016). This information would be of valuable importance considering conservation biology at the Kerguelen Islands, because it would allow to fully understand the impacts of *M. soledadinus* on native and introduced prey species.

Individuals from front populations have increased starvation tolerance and increased locomotor activity which, combined with the year-round activity of this ground beetle, suggest that farther locations should be colonised. A sustained metabolic activity could be expected in edge

populations, but was not readily observed (**Chapter 3**). Metabolic improvement, which could enhance growth rates, has been described in several range expanding taxa (Bøhn et al., 2004; Haag et al., 2005; Lombaert et al., 2014; Therry et al., 2014; Myles-Gonzalez et al., 2015). The efficiency of metabolic improvement could be maximised if the amount of food resources that are consumed can be increased. Consistently, Lindström et al. (2013) reported higher feeding rates in edge population of *Rhinella marina* compared with specimens from core populations. At present, we cannot rule out the possibility that core, intermediate and range margin populations differ in their feeding behaviour as a function of resource availability and abundance of both prey and predators. This aspect was not presented in the present dissertation, even though an experiment has been conducted to address this objective already. We compared functional responses of six populations of *M. soledadinus* sampled along the invasion gradient. Cross comparisons of per capita feeding rates were obtained, with different densities of both prey and predator; Médoc et al., 2013).

d) *Range expansion of* M. soledadinus*: a conservation issue*

We have explored the ecology of *M. soledadinus* at the Kerguelen Islands, and considered most of the field-studies as natural experiments that allow to highlight ecological and evolutionary processes associated with range expansion and responses to novel environmental conditions. Altogether the collected data suggest that the range of expansion of *M. soledadinus* will continue to increase at the Kerguelen Islands. Biocontrol procedures (entomopathogen fungi for example) are, at present, not possible for this species, and would require further introduction, which is prohibited in this context of nature reserve. Prevention might be, at least for the next decades, the best way to deal with the expansion of *M. soledadinus*. Biosecurity measures are installed at the French subantarctic territories since 2013, which was most probably too late for the control of the range expansion of this introduced alien species. Yet, despite being lately implemented, these biosecurity measures are probably efficient for preventing human-assisted range shifts of this species from the Kerguelen Islands to other subantarctic islands. Indeed, Laparie & Renault (2016) recently suggested that the introduction risk to Saint Paul and Amsterdam islands is really high, especially because the logistic ship 'Marion Dufresne' sails from the Kerguelen Islands to over there.

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Résumé en Français

Caractérisation phénotypique et génétique du carabique invasif *Merizodus soledadinus* aux Iles Kerguelen

Pour mes parents…

Introduction

La distribution des espèces varie naturellement, en raison de modifications climatiques ou d'interactions biotiques. Depuis la révolution industrielle et l'explosion de la démographie humaine, le transport et l'introduction par l'homme de propagules hors de leur aire de répartition s'est multiplié, entrainant un nombre croissant d'exemples de communautés natives dominées par des populations d'espèces arrivées récemment. Les invasions biologiques sont désormais comprises dans le top 5 des menaces qui pèsent sur la biodiversité, aux côtés du changement climatique, de la fragmentation des habitats et du changement d'usage des sols, et participent à l'homogénéisation de la biodiversité.

L'intérêt des chercheurs pour les invasions biologique fut principalement initié par le lancement du programme SCOPE en 1982. En premier lieu, l'étude des espèces invasives est motivée dans un but de conservation (les comprendre pour les gérer), mais d'autres domaines de recherche se sont focalisés sur les invasions biologiques parce qu'elles constituent des expériences naturelles idéales pour étudier en temps réel les processus éco-évolutifs, comme le changement de niche écologique, les proliférations, l'accélération du front d'expansion. Il résulte de cet intérêt un nombre aujourd'hui pharamineux d'études de cas d'invasions biologiques, qui sont menées à l'aide de méthodes différentes, dans des objectifs différents, sur une grande variété de modèles taxonomiques. Ce foisonnement a donné lieu à l'émergence de concepts et de terminologies parallèles et synonymes (non-native, introduite, exotique, établie, invasive…). Des cadres théoriques généraux ont vu le jour ces dix dernières années afin de clarifier le débat, et afin de généraliser et unifier les concepts relatifs aux invasions biologiques.

1) Le processus d'invasion

Williamson et Richardson ont travaillé à l'élaboration de concepts généraux, sur les invasions d'animaux et de plantes respectivement. Leurs travaux ont permis d'établir que le processus d'invasion est une séquence d'étapes ou de barrières auxquels les propagules sont confrontées. Les trois stades principaux sont la phase de transport, pendant laquelle les propagules prélevées dans l'aire native doivent survivre au transport vers l'aire d'introduction, puis la phase d'établissement, pendant laquelle les propagules introduites doivent réussir à survivre et se reproduire de manière à installer une population pérenne (ce stade est souvent associé à une période de latence, parce que l'établissement d'une population viable peut nécessiter de multiples introductions et/ou une période d'adaptation aux

nouvelles conditions environnementales rencontrées). Enfin, la troisième et dernière phase, l'invasion, pendant laquelle la population établie et pérenne va proliférer et étendre son territoire. Cette dernière phase est souvent rapide et exponentielle.

Etant donné l'intensité actuelle du commerce global, le nombre probable d'introductions doit être très élevé. Cependant, peu de propagules introduites conduisent à l'établissement d'une population viable, et encore moins deviennent invasives. La force des contraintes imposées par les filtres environnementaux déterminants le succès des espèces invasives a été illustré par la Règle des 10 de Williamson (1996) : sur 100 espèces introduites, 10 réussiront probablement à établir une population viable et une seulement risque de devenir invasive.

2) Invasibility et invasiveness

La recherche sur les espèces invasives et le processus d'invasion s'est longtemps penché sur la caractérisation générale de traits partagés par les espèces invasives et/ou par les environnements envahis afin d'essayer de prédire l'émergence d'invasions biologiques. Par exemple, les caractéristiques d'un « bon envahisseur » rassemblent des traits d'histoire de vie favorisant une reproduction rapide, un large régime trophique, de fortes capacités de compétition. Les environnements sensibles aux invasions biologiques sont souvent caractérisés par des communautés natives peu diversifiées. En effet, dans un environnement 'insaturé', la probabilité pour une nouvelle espèce d'établir une population viable dans une niche écologique sous-occupée est plus forte (si les autres conditions biotiques ou abiotiques le permettent). Dans ce contexte, les écosystèmes insulaires apparaissent particulièrement sensibles aux invasions biologiques, de par le fréquent fort taux d'endémicité de leurs communautés indigènes. D'autre part, la perturbation des milieux est généralement reconnue comme un facteur facilitant l'établissement d'espèces non natives, et la question est souvent posée de savoir si les espèces invasives ne seraient que des « passagers » du changement global, des « symptômes » de l'affaiblissement des communautés introduites. La recherche de traits généraux partagés par les espèces invasives et les environnements envahis est souvent peu concluante et faiblement applicable : en effet les travaux qui ont exploré ces hypothèses ont dévoilé la grande variété de stratégies invasives, dépendant à la fois des caractéristiques des espèces et des habitats étudiés. Le succès invasif est aujourd'hui vu comme la correspondance entre une population et un système.

3) L'apport des biologistes évolutifs

Un peu plus tardivement que les écologues, les évolutionnistes se sont finalement aussi intéressés aux invasions biologiques. En effet, ces expériences naturelles permettent d'étudier l'adaptation et la réponse à de nouvelles pressions de sélections à une échelle temporelle écologique, et non géologique. Malgré l'intérêt récent porté sur le sujet par les biologistes de l'évolution, des avancées majeures ont été réalisées vers la compréhension des changements évolutifs durant les invasions. Le développement

récent d'outils moléculaires qui permettent le séquençage à haut débit et à prix raisonnable a permis l'accès à ces techniques pour des organismes non model, ce qui est souvent le cas pour les populations invasives.

Les outils moléculaires sont beaucoup utilisés pour reconstruire les routes d'invasions (les vecteurs de transport) et l'origine des populations invasives. Ces études, couplées à des données historiques, constituent des outils puissants pour mieux appréhender les origines des populations envahissantes et constituent une base solide pour tester des hypothèses éco-évolutives entre les populations natives et introduites.

Les populations introduites sont le plus souvent fondées par un très petit nombre de propagules, qui portent une fraction réduite de la diversité génétique initiale (population d'origine), et sont souvent caractérisées par de forts taux de consanguinités. La diversité génétique constitue le substrat du potentiel adaptatif des espèces, qui permet l'adaptation à de nouvelles pressions de sélection. Le paradoxe génétique des invasions pose le problème suivant : comment les espèces invasives s'adaptent aux nouvelles conditions rencontrées malgré une diversité génétique réduite ? La comparaison des niveaux de diversité entre populations natives et introduites révèle des résultats contrastés : si de nombreuses études ont démontré des niveaux réduits de diversité génétique dans les populations introduites comparées aux populations natives des mêmes espèces, des niveaux supérieurs de diversité ont également été mis en évidence. Ces patrons sont attribués à des phénomènes d'« *admixture* », de mélange de diversité génétique originaire de différentes populations sources dans le cas d'introductions multiples. Les goulots d'étranglement génétiques (« *bottlenecks* ») ne semblent pas contraindre le succès invasif ni même l'adaptation rapide des espèces introduites. Des mécanismes moléculaires peuvent assister le potentiel invasif des espèces, comme des inversions chromosomiques (mises en évidences dans le cas de l'invasion du continent américain par *Drosophila subobscura*) ou comme l'hybridation intra- ou interspécifique, qui peut entrainer la formation de phénotypes transgressifs (à la fitness meilleure que celles des deux parents, c'est par exemple le cas chez les spartines).
L'adaptation locale et parfois rapide des espèces introduites fait l'objet de questionnements au sein de la communauté des biologistes évolutifs, à savoir sur quel substrat de variabilité génétique la sélection naturelle agit-elle dans les cas de faible diversité génétique. La piste de la variation génétique stagnante (« *standing genetic variation* ») est favorisée au profit de l'apparition de nouvelles mutations, parce que ces dernières ont des probabilités plus réduites d'être bénéfiques et d'atteindre des fréquences suffisantes pour se fixer.

Une hypothèse alternative pour expliquer l'adaptation locale des espèces introduites dans les cas de faible diversité génétique serait la plasticité phénotypique, qui confèrerait de bonnes performances dans une large gamme de conditions environnementales. Cette plasticité étant couteuse, il est probable

qu'elle ne soit bénéfique que pendant les premiers stades de l'invasion, pour ensuite disparaitre au profit de la fixation d'allèles adaptés localement.

4) La dispersion au front d'invasion

La phase d'expansion géographique caractérise les espèces invasives, qui ne sont parfois détectées qu'à ce stade du processus d'invasion. Les capacités de dispersion des individus déterminent la vitesse de colonisation de nouveaux habitats. Le processus d'expansion combine des évènements de dispersion à petite distance et quelques évènements de dispersion à longue distance, constituant une succession de fondations. Les individus qui dispersent le plus loin ont théoriquement des avantages directs en termes de fitness, en raison des pressions de compétition moindre dans les milieux colonisés par peu d'individus contrairement aux populations densément peuplées de la population d'origine. Le front d'invasion est donc constitué d'habitats colonisés en premier lieu par les individus disposant des meilleures capacités de dispersion. En se reproduisant entre eux, au fur et à mesure de l'avancée du front il peut se produire un effet de « Village Olympique », où les descendants des populations du front d'invasion sont dotés de capacités accrues de dispersion (si les traits de dispersion sont héritables). La répétition de ce phénomène entraine, au fil des générations, une différentiation phénotypique entre les populations anciennement établies et le front d'expansion. Ce phénomène est appelé le tri spatial.

5) Présentation du système étudié : un carabique aux Iles Kerguelen

Les îles subantarctiques sont situées entre 45° et 54° de latitude sud et constituent la majeure partie des écosystèmes terrestres dans cette région du globe. La gouvernance de ces îles se partage entre le Royaume Uni, l'Afrique du Sud, la France, l'Australie et la Nouvelle Zélande. Ces îles sont de taille variable, quasiment toutes volcaniques, et partagent des similarités en terme de conditions climatiques (forte influence du vent, beaucoup de précipitations, faibles variabilités saisonnières, température annuelle moyenne entre 1.5 et 10°C) et de biotopes (faible diversité d'espèces et de groupes fonctionnels, absence d'arbres). La faune terrestre est dominée par la communauté d'arthropodes, la plupart des espèces étant des décomposeurs, concentrés au bord de mer auprès des colonies d'oiseaux et de mammifères marins qui viennent muer et se reproduire. Ces colonies constituent la majeure partie des intrants de nutriments dans l'écosystème terrestre des îles subantarctiques, expliquant une concentration d'espèces près du rivage. Les îles subantarctiques abritent une forte proportion d'espèces aptères par rapport à d'autres systèmes (insulaires ou continentaux), probablement à cause de l'environnement à la fois froid et venteux, où la fonction de vol est désavantagée.

Les îles subantarctiques ne sont pas habitées et ont une histoire anthropique récente et tournée vers la mer (découvertes fin du XVIème siècle, longue période baleinière à partir du milieu du XIXème). Certaines îles sont très rarement visitées, principalement par des scientifiques, et demeurent des sanctuaires (comme par exemple l'île aux Cochons de l'archipel de Crozet) ; sur d'autres îles des

stations de recherches, peuplées pendant l'été austral ou à l'année, ont été bâties dans les années 1950. Malgré leur isolement géographique extrême et la courte histoire anthropique, les îles subantarctiques abritent de nombreuses espèces introduites, parfois surpassant les effectifs de diversité spécifiques natives. Les relevés climatiques à long termes (>50 ans) montrent que ces îles sont également soumises à un rapide changement climatique, se traduisant par des hivers moins froids et une diminution des précipitations.

Les îles subantarctiques constituent des laboratoires à ciel ouvert, qui permettent d'étudier les processus écologiques dans des conditions de faible impact anthropique (excepté pour le changement climatique), au sein de chaines trophiques réduites à faible redondance fonctionnelle. La diversité spécifique réduite ainsi que la surface restreinte de ces îles favorisent le suivi à long terme des populations ainsi que la détection d'espèces nouvellement introduites. Outre les avantages indéniables que ces caractéristiques confèrent à la protection et à la conservation de ces milieux, elles permettent également d'étudier les mécanismes et la dynamique associés aux invasions biologiques, tels que l'expansion géographique, les impacts de nouveaux prédateurs ou compétiteurs sur les communautés natives, les modifications post-introduction. Etant donné la faible étendue des impacts anthropiques sur ces écosystèmes, l'impact direct le plus notable de l'activité humaine sont les espèces invasives. Les politiques de conservation ont entrainé la mise en place de tests d'éradication sur différentes îles et ilots, ce qui permet d'étudier à long terme les processus de recolonisation et les mécanismes de résilience dans ce contexte subantarctique. Les îles subantarctiques sont des sites majeurs de l'étude aujourd'hui cruciale des effets combinés des invasions biologiques et du changement climatique.

Merizodus soledadinus est un carabique prédateur natif de la Patagonie et des îles Falklands. Il a été observé pour la première fois en Géorgie du Sud en 1982, et à Kerguelen en 1939. Les données historiques suggèrent que l'introduction de cette espèce à Kerguelen résulte d'un unique évènement, lorsque les frères Bossières expérimentèrent un élevage de moutons, notamment originaires des Falkland, sur le site de Port Couvreux. Lorsque René Jeannel observe *Merizodus soledadinus* en 1939, il ne le trouve qu'autour des baraquements abandonnés de la bergerie. Depuis 1939, la distribution de cette espèce sur l'archipel est régulièrement suivie, et ces données fournissent des informations précises sur la dynamique d'expansion géographique de cette espèce, et notamment sur les temps de résidence des différents sites colonisés le long du gradient d'invasion. Des études ont d'ailleurs montré une relation négative entre ce temps de résidence et les abondances d'espèces de diptères natives, suggérant un fort impact de prédation de *M. soledadinus* sur les communautés natives d'arthropodes. Une étude récente a mis en évidence que les populations récemment établies étaient composées d'individus plus gros que les populations anciennement établies, suggérant une possible différentiation phénotypique le long du gradient d'invasion entrainée par un tri spatial.

Cette thèse a pour but de contribuer à l'explication du succès invasif de *M. soledadinus* aux îles Kerguelen, à travers l'utilisation d'une combinaison de méthodologies alliant l'autécologie classique, la génomique, la métabolomique et le comportement.

Chapitre 1 – Les adultes de *M. soledadinus* vivent longtemps, sont actifs à toutes les saisons et il n'y a pas de période de ponte

Contexte : cette étude vise à améliorer nos connaissances sur l'autécologie de cet insecte aux Iles Kerguelen.

Méthodologie : la durée de vie des adultes a été déterminée grâce au maintien en conditions contrôlées de 500 individus prélevés juste après leur émergence (cuticule rouge). Un proxy de la fécondité a été estimé en disséquant des femelles prélevées tous les mois sur une période de 18 mois et en comptant les œufs. La quantité de réserves a été estimée au cours des mois grâce à des analyses colorimétriques. L'activité des individus au cours de l'année a été analysée à partir d'un jeu de données rassemblant 10 ans de piégeage à proximité de la base de Port-aux-Français (pots barber, 2005 - 2015).

Résultats : les adultes *M. soledadinus* vivent en moyenne 8 mois, mais leur longévité peut s'étendre jusqu'à plus de 14 mois en conditions contrôlées. Les femelles disposent en moyenne de 8 œufs dans leurs ovarioles, de manière constante au cours des mois, sans période notable de ponte. Les données de piégeage montrent que les adultes sont actifs toute l'année, avec un léger pic de capture pendant l'été austral. Les réserves corporelles restent relativement stables au cours de l'année, suggérant que les individus continuent à se nourrir pendant l'hiver, potentiellement avec des proies différentes qu'en été.

Conclusion : ces résultats sur l'écologie de *M. soledadinus* aux Iles Kerguelen permettent de mieux appréhender les traits d'histoires de vie de cet insecte invasif. Même si le cycle de vie complet reste inconnu, les densités rencontrées sur le terrain suggèrent un fort impact sur les communautés d'arthropodes de ce vorace préateur.

Chapitre 2 – les analyses génomiques révèlent la faible diversité génétique et l'absence de structure des populations de *M. soledadinus* aux Iles Kerguelen

Hypothèse 1 : l'introduction unique d'individus fondateurs a probablement engendré un fort goulot d'étranglement et une diversité génétique amoindrie chez les populations de *M. soledadinus* des Iles Kerguelen comparé aux populations natives.

Hypothèse 2 : l'expansion géographique naturelle, à partir de Port Couvreux, a probablement entrainé un tri spatial des individus, ce qui pourrait être détecté par un décroissance des niveaux de diversité génétique à partir du site d'introduction vers le front d'invasion.

Méthodologie : une approche moléculaire de RAD sequencing a été testée et s'est révélée efficace sur notre modèle biologique. Cette technique consiste à séquencer un sous-échantillonnage du génome correspondant aux régions flanquantes (300 paires de bases) de sites de restrictions spécifiques. Ces sites de restrictions sont distribués le long du génome, et le génotypage de sites polymorphes dans ces séquences permet d'estimer la diversité génétique des individus séquencés. Deux jeux de données ont été créés : un premier jeu de données regroupe des individus originaires de Patagonie, de Géorgie du Sud et de Port Couvreux (Kerguelen), et permet de reconstruire une partie de la biogéographie de cette espèce, et de comparer les diversités génétiques des populations natives et introduites. Le second jeu de données regroupe des individus échantillonnés dans 10 populations le long du gradient d'invasion de *M. soledadinus* sur l'île principale des Kerguelen, dans le but d'étudier la génétique des populations à Kerguelen.

Résultats : la diversité génétique des individus de Kerguelen est réduite par rapport aux populations de Géorgie du Sud et de Patagonie, confortant l'hypothèse d'un unique évènement d'introduction. Les analyses de structure montrent que les individus de Géorgie du Sud et de Kerguelen sont plus proches entre eux comparé aux individus de Patagonie. Nous n'avons pas trouvé de structure de population le long du gradient d'invasion à Kerguelen. La population la plus récente échantillonnée montre un déficit en hétérozygotes, et la population d'Estacade, particulièrement homogène est probablement le résultat d'une introduction d'origine anthropique.

Conclusion/perspectives : Afin de finaliser cette étude et de pouvoir valoriser ces résultats, des individus complémentaires doivent être séquencés : des individus des Falkland pour compléter la biogéographie de l'espèce, et des individus de la station de recherche de Port aux Français à Kerguelen afin de vérifier l'existence d'introductions assistées par l'homme, depuis la base.

Chapitre 3 – la divergence phénotypique entre les populations anciennement et récemment établies suggère une dynamique de tri spatial

Objectifs : cette étude vise à poursuivre la mise en évidence du gradient morphologique établis au cours de l'expansion géographique, par la caractérisation de nombreux traits (morphologiques, physiologiques, comportementaux) liés à la dispersion.

Méthodologie : des individus ont été échantillonnés dans 8 sites caractérisés par différents temps de résidence le long du gradient d'invasion. Ils ont été phénotypés pour les traits suivants : morphologie, capacités de tolérance de jeûne, activité locomotrice, propension à disperser, activités enzymatiques impliquées dans les voies du métabolisme énergétique et quantification en composés circulants (GC-TOF-MS).

Résultats : confirmant les études précédentes, les individus échantillonnés dans des populations du front d'invasion sont plus gros. Ils sont également plus actifs, résistent mieux au jeûne et présentent des empreintes métaboliques distincts des individus échantillonnés dans des populations plus

anciennes. Les activités enzymatiques sont relativement similaires entre toutes les populations et la propension à disperser ne montre pas de différences selon le temps de résidence des populations.

Conclusion: Cette étude, par une approche intégrative de traits phénotypiques, permet de démonter l'évidence d'une différentiation phénotypique au cours de l'invasion, entre les populations du front et les populations anciennement établies, suggérant l'implication de tri spatial.

Chapitre 4 – l'humidité relative et l'accessibilité de ressources en eau déterminent l'établissement des populations de *M. soledadinus.*

Contexte : *M. soledadinus* est distribué aux Iles Kerguelen le long des côtes et des cours d'eau, et on peut se demander quels facteurs environnementaux gouvernent les exigences écologiques relatives à l'habitat, entre la disponibilité en eau et en ressources trophiques.

Méthodologie : des individus ont été exposés à trois conditions d'humidité relative (30%, 70% et 100%), leur survie a été mesurée ainsi que la dynamique de perte d'eau corporelle et les modifications métaboliques dans ces trois conditions. En parallèle, des individus ont été isolés et privés de nourriture.

Résultats : *M. soledadinus* est très tolérant à de longues périodes de jeûne, mais survivent comparativement très peu de temps lorsqu'ils sont exposés à des conditions moyennes et fortes de stress hydrique. La quantité d'eau corporelle décroit rapidement dans ces conditions, et les analyses métaboliques révèlent le déclin rapide de la physiologie des insectes.

Conclusions/perspectives : les résultats indiquent que la distribution géographique des populations de *M. soledadinus* aux Iles Kerguelen est principalement contrainte par la disponibilité en eau.

Chapitre 5 – les habitats d'altitude sont contraignants pour *M. soledadinus,* mais sont tout de même colonisés

Contexte : depuis les années 2000 l'expansion géographique de *M. soledadinus* aux Iles Kerguelen comprend la colonisation d'habitats en altitude (jusqu'à 400m), probablement assisté par le changement climatique. Cette étude s'intéresse aux effets morphologiques et physiologiques de la vie en altitude.

Méthodologie : des individus ont été échantillonnés le long de deux transects altitudinaux (0 à 250m) et le long d'un transect plat, ce dernier servant à discriminer les effets de la diminution en température des effets de la raréfaction de ressources trophiques associées à l'éloignement à la côte. La morphologie et la physiologie (composés de réserve, métabolites circulants) de ces individus ont été analysées.

Résultats : les individus échantillonnés le long des transects altitudinaux montrent une décroissance de la taille corporelle, qui peut être attribuée aux conditions thermiques suboptimales en altitude lors du développement larvaire. La quantité de glycogène est constante le long des trois transects, les femelles échantillonnées loin de la côte ont des quantités amoindries en protéines et la quantité de

triglycérides tend à augmenter chez les individus prélevés en altitude. Les analyses métaboliques permettent de discriminer les échantillons prélevés au bord de la mer (effets de l'environnement marin) des échantillons prélevés en altitude, au-dessus de 200m. Ces derniers montrent des patrons métaboliques semblables à ceux d'insectes exposés au froid.

Conclusion : les conditions thermiques des habitats d'altitude sont contraignantes, mais le changement climatique semble permettre la colonisation de ces habitats.

Discussion générale

1) *Les avantages de ce modèle biologique*

L'invasion des îles Kerguelen par le carabe *M. soledadinus* constitue une opportunité pour étudier les processus éco-évolutifs et les mécanismes sous-jacents durant l'expansion géographique. En effet, ce cas présente une combinaison rare des caractéristiques telles que l'introduction probablement unique, à partir d'une origine conne, dans un site unique (Port Couvreux).
Les résultats des analyses génétiques présentées dans le chapitre 2 corroborent l'hypothèse de l'évènement unique d'introduction, et confirment la chronoséquence de l'expansion géographique issue du suivi à long terme de la biodiversité terrestre.

Les résultats accumulés sur l'écologie de cet insecte invasif représentent des connaissances essentielles pour comprendre la dynamique des populations, les routes potentielles d'invasion et délimiter la niche écologique de cette espèce. Ces données pourraient notamment permettre de paramétrer un modèle d'expansion afin de prédire la colonisation de l'archipel par cette espèce et proposer des mesures de gestion. En combinant les données de distribution, écologiques et génétiques, il serait possible dans un futur proche de construire une matrice de résistance qui mettrait en évidence les habitats les plus favorables et les chemins de moindre coûts entre eux à l'échelle des îles Kerguelen. Un modèle préliminaire inclurai la topographie, parce que *M. soledadinus* est trouvé jusqu'à 400m d'altitude, et que les habitats d'altitude sont contraignants (chapitre 5). De plus, la distribution littoral et le long de cours d'eau, ainsi que la faible tolérance à la dessiccation indiquent un fort coût des habitats éloignés de la côte et du réseau hydrographique.

Aux îles Kerguelen, *M. soledadinus* n'a pas de compétiteur. En revanche, de forts taux de compétition intra-spécifiques sont attendus dans les localités à fortes densités. Des interactions cannibales ont été observées en conditions contrôlées entre adultes et entre adultes et larves. En théorie, la compétition de parentèle est une force majeure pouvant déclencher la dispersion chez certaines espèces. Les analyses du chapitre 2 n'ont pas permis de mettre en évidence de structure génétique le long du gradient d'invasion, ce qui pourrait être expliqué par un faible nombre d'individus fondateurs (en 1913). Si cette hypothèse est vraie on peut d'attendre à de forts degrés de parentés entre les individus, et à une augmentation de ce degré de parenté dans les populations du front d'invasion, en raison des

différents évènements fondateurs depuis le site de Port Couvreux. Des reconstructions de parentèle sont donc requises afin de valider ou d'infirmer cette hypothèse.

Merizodus soledadinus a été accidentellement introduits aux îles Kerguelen mais aussi en Géorgie du Sud. Ces deux îles subantarctiques partagent des conditions climatiques similaires avec l'aire native de cet insecte (pré-adaptation). Ces cas « répliquées « d'invasions constituent un terrain fertile pour la mise en place d'études comparatives des patrons écologiques et évolutifs impliqués dans l'introduction et l'expansion géographique. Cette situation permettrait notamment de tester la prédiction théorique selon laquelle la dynamique d'expansion est réduite si l'espèce focale est en compétition avec une ou plusieurs autres espèces. Or, en Géorgie du Sud, *M. soledadinus* est en compétition avec le carabe *Trechisibus antarcticus*. Des études de l'écologie de ces deux milieux permettraient de comparer et de quantifier les effets de la compétition intra et inter-spécifiques sur la dynamique de l'expansion géographique.

2) Les limites du modèle biologique

1/ Où sont les larves ?

Des lacunes importantes restent à combler dans la compréhension de la biologie de la reproduction et le cycle de vie de cet insecte, et particulièrement des stades larvaires : les besoins trophiques et abiotiques, les taux de recrutement, les pressions de prédation/compétition, les capacités de mouvement. La compréhension du cycle de reproduction constitue cependant un verrou essentiel pour appréhender la dynamique des populations. De plus, le control de la reproduction *via* l'élevage permettrait d'étudier l'héritabilité des caractères, en particulier ceux pour lesquels des variations ont été mises en évidence (chapitre 3) entre les populations du front d'invasion et les populations anciennement établies.

2/ Déterminer l'âge

Les résultats des différentes expériences sont soumis à la variabilité inter-individuelle du sexe, du statut trophique et de l'âge des individus échantillonnés. Les protocoles expérimentaux ont été conçus pour atténuer ou prendre en compte ces biais, excepté pour l'âge, qui demeure incertain. L'âge des individus pourrait être estimé en formant des classes d'âge à partir d'indices morphologiques tels que l'usure mandibulaire ou des tarses, ou via la quantification d'hydrocarbones cuticulaires qui varient selon l'âge.

3/ Régime trophique

Le régime trophique de *M. soledadinus* a été évalué par des expériences de choix de proies potentielles en conditions contrôlées. Une analyse précise du régime requière des analyses de metabarcoding du contenu du tractus digestif.

Les résultats du chapitre 3 montrent une augmentation de la résistance au stress trophique et de l'activité locomotrice des individus du front d'invasion. Combinés à l'activité annuelle continue de cet

insecte, ces résultats suggèrent la poursuite de la colonisation de sites de plus en plus éloignés. Il est possible que le comportement de nutrition change selon le temps de résidence des populations, avec des besoins énergétiques plus forts des populations au front d'invasion comparé aux populations intermédiaires et anciennement établies, en fonction de la disponibilité en nourriture et de l'abondance en proies et prédateurs. Pour répondre à cette question, une expérience de comparaison de la réponse fonctionnelle d'individus issus de 6 populations le long du gradient d'invasion a été réalisée (résultats en cours d'analyse, non présentés ici).

3) Expériences menées pendant la thèse mais non inclues dans le manuscrit de thèse

1/ Reconstruction de parenté

Cette expérience vise à étudier la structure de parenté à fine échelle géographique, afin de mieux comprendre la dynamique locale de dispersion. Ainsi, 639 adultes ont été échantillonnés le long d'un transect de 1500m parallèle à la côte à Cataractes, et ont été génotypés à 13 loci microsatellites. Les relations de parentés seront extraites à l'aide du logiciel Coancestry.

2/ Réponse aux stress

La théorie prédit qu'au cours de l'expansion géographiques, les individus du front d'invasion auront des réponses au stress différentes des populations plus anciennement établies en raison de leur plus fréquente exposition à des conditions environnementales nouvelles. Ainsi, des individus échantillonnés dans 6 populations de long du gradient d'invasion ont été exposés à des conditions stressantes : dessiccation, jeûne, régime thermique fluctuant chaud et les trois stress en même temps. Cette expérience est toujours en cours à Port-aux-Français.

3/ Colonisation à Isthme Bas

Les sites les plus récemment colonisés bénéficient d'une estimation précise de la date d'arrivée et de l'établissement de *M. soledadinus*. A Isthme Bas, cette arrivée a même été anticipée avec la pose de pièges barber en 2005, et l'arrivée sporadique des premiers individus entre 2006 et 2010. A partir de ces échantillons, il sera possible de caractériser les premiers colonisateurs au front d'invasion (sexe, quantité de réserves corporelles, charge en œufs des femelles, âge éventuellement).

Pour conclure, les données collectées sur l'écologie de *M. soledadinus* à Kerguelen suggèrent que la colonisation va se poursuivre. Les procédures de bio-contrôle (champignons entomopathogenes) sont inenvisageables, et la prévention reste le meilleur moyen de gérer cette invasion. Les mesures de biosécurité instaurées en 2013 sont probablement efficaces pour éviter le transport assisté par l'homme au sein de l'archipel, mais aussi de Kerguelen vers Amsterdam et St Paul, où le Marion Dufresne passe juste après Kerguelen au cours de sa rotation.

ABSTRACT
Global trade and human movements increase the likelihood of long-distance transportation of propagules and their subsequent introduction into new geographic regions. In some instances, newly established species can become dominant in invaded communities, at the expense of native species. Besides threatening invaded communities and ecosystem functions, biological invasions constitute natural experiments that allow to study eco-evolutionary processes in real time, including the occurrence of new biotic interactions affecting community composition, rapid adaptation to novel environmental conditions, or dispersal evolution at range margins. Because of their impoverished native communities, oceanic islands' ecosystems are particularly sensitive to biological invasions, and the French subantarctic islands are no exception. For instance, the flightless predatory carabid beetle *Merizodus soledadinus* is native from the southern tip of South America, and has been accidentally introduced to the Kerguelen Islands in 1913. In the present work, we aimed at understanding the main mechanisms underlying the invasive success of this insect at the Kerguelen Islands. Using a vast array of methodologies, ecological features of *M. soledadinus* were investigated with analytical procedures scaling from population to molecule through the individual level. Genetic investigations support the historically-based hypothesis of a single introduction event at a unique location of the Kerguelen Islands. No genetic structure was observed among individuals sampled from different populations along the invasion gradient. We tested the hypothesis of spatial sorting of populations during range expansion, by exploring phenotypic changes among individuals sampled along the invasion gradient. The measured phenotypic traits revealed major differentiation of adults according to the residence time of their populations, confirming the occurrence of spatial sorting of populations during geographic expansion. We also demonstrated that the geographic expansion of *M. soledadinus*, and microhabitat selection, are primarily governed by the availability of water resources, as suggested by the high sensitivity to water stress of adults of this ground beetle. In parallel, colonisation of altitudinal habitats is governed by thermal conditions, which seem to be physiologically constraining from 200m asl onwards. As the altitudinal distribution of *M. soledadinus* still extends, we concluded that ongoing climatic changes play a pivotal role in this expansion. Finally, adults of this ground beetle are long-lived and active year-round. The ecological knowledge of *M. soledadinus* characteristics and spatial expansion dynamics suggest that the colonisation process of the Kerguelen archipelago by this species will continue. Altogether, these data could be used for parametrising range expansion models that would delineate dispersal pathways and expansion rates, in the objective to assist stakeholders' management decisions.

Keywords: Biological **I**nvasions, **D**ispersal **E**cology, **E**cophysiology, **P**henomics, **P**opulation **G**enetics

RÉSUMÉ
Le commerce mondial et les mouvements humains accroissent les probabilités de transport à longue distance de propagules, et leur introduction dans de nouvelles aires géographiques. Dans certains cas, des espèces récemment établies peuvent devenir dominantes dans la communauté envahie. Malgré les menaces sur les communautés natives et le fonctionnement des écosystèmes, les invasions biologiques constituent des expériences naturelles qui permettent d'étudier les processus éco-évolutifs en temps réel, notamment l'impact de nouvelles interactions biotiques sur la composition et la dynamique des communautés, l'adaptation rapide à de nouvelles conditions environnementales, ou la dispersion en limite de répartition. Les îles océaniques sont particulièrement sensibles aux invasions biologiques en raison de la faible diversité de leurs communautés natives. Dans les terres australes françaises, le carabique marcheur *Merizodus soledadinus*, natif de Patagonie, a été accidentellement introduit à Kerguelen en 1913. La présente étude vise à comprendre les principaux mécanismes à l'origine du succès invasif de cet insecte aux Iles Kerguelen. Un large ensemble de méthodes ont été utilisées pour explorer les traits écologiques de *M. soledadinus*, des populations à la molécule. Les analyses génétiques confortent l'hypothèse historique d'un unique évènement d'introduction dans un seul site des Iles Kerguelen. Les populations échantillonnées le long du gradient d'invasion ne montrent pas de structuration génétique. Les traits phénotypiques mesurés montrent une forte différentiation entre les individus selon le temps de résidence des populations, confirmant l'hypothèse de tri spatial des populations au cours de l'expansion géographique. Nous avons démontré que l'expansion géographique et la sélection d'habitats par *M. soledadinus* est principalement gouvernée par la disponibilité en eau, comme le suggère par la forte sensibilité des adultes au stress hydrique. En parallèle, la colonisation d'habitats en altitude dépend des conditions thermiques, qui semblent être contraignantes pour cet insecte à partir de 200m d'altitude. La colonisation d'habitats d'altitude progresse pourtant, probablement assistée par le changement climatique. Pour finir, les adultes *M. soledadinus* sont longévifs et actifs toute l'année. Les connaissances apportées sur l'écologie de *M. soledadinus* et sur la dynamique de son expansion géographique suggèrent la poursuite de la colonisation de l'archipel par ce prédateur. L'ensemble de ces connaissances pourraient être utiles à la paramétrisation d'un modèle d'expansion géographique, qui permettrait de définir les routes de dispersion et les taux d'expansion, dans l'objectif d'assister les mesures de gestion par les agents de la Réserve naturelle des Terres Australes et Antarctiques Françaises.
Mots-clefs : Ecologie de la **D**ispersion, **E**cophysiologie, **G**énétique des **P**opulations, **I**nvasions **B**iologiques **P**hénomique