

Comparing thermal tolerance across contrasting landscapes: first steps towards understanding how landscape management could modify ectotherm thermal tolerance

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Abstract. 1. Insects are highly dependent on ambient temperatures to ensure their biological functions. Their persistence in the environment and their resistance to unfavourable temperatures are governed by their physiological thermal tolerance.

2. Global change extends beyond climatic conditions to encompass modifications to the landscape. Studies of climate change and landscape composition effects on ecosystem services, such as biological control, are commonly performed independently. Moreover, coarse scales are not always relevant when assessing climate change's impacts on ectotherms. We aimed to better understand the ecological relationships that may exist between microclimatic variation and insect thermal tolerance across a landscape composition gradient.

3. To determine how landscape composition may impact insect thermal tolerance, parasitic wasps (Hymenoptera: Braconidae: Aphidiinae) of aphids were sampled along a landscape gradient from 'closed' to 'open' habitats. Sampling was performed during the winter 2013/14 and spring 2014 in cereal fields of Brittany, France. Meteorological data were recorded along this gradient.

4. First, our results show an influence of landscape composition on local microclimate. Additionally, parasitoids from open landscapes had a higher tolerance to low temperatures, leading to higher physiological costs, compared with parasitoids from closed landscapes. This trend was stronger in winter than in spring.

5. These results have numerous implications in the context of climate change, suggesting that targeted landscape management practices could create sheltered microclimatic areas that reduce the physiological costs of thermal tolerance and promote the persistence of biological control agents.

Key words. Aphidiinae, *Aphidius*, biological pest control, Braconidae, climate change, conservation ecology, critical temperatures, Hymenoptera, insect ecophysiology, landscape ecology, thermal biology.

Introduction

Climate models predict a global average temperature increase of between 1.7 and 6 °C by the year 2100 (IPCC,

2013). In addition, an increase in the frequency of extreme events (heat or cold spells) is expected, especially in temperate regions (Hance *et al.*, 2007). Meanwhile, European landscapes have undergone increasing homogenisation since the 1950s due to agricultural intensification, leading to the removal of hedges, groves, woods, and natural grasslands (Millennium Ecosystem Assessment, 2005). This homogenisation has reduced the effectiveness of

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ecosystem services, such as pest control (Thies *et al.*, 2011). Although the combined influences of landscape properties and climate change (warming winters, increased incidence of cold spells) on ecosystems are still poorly understood, there is increasing evidence that working on both of these aspects could yield more important insights than considering them separately.

Despite numerous reports of climate change impacts, the gap between the coarse scale at which climate data are collected and the climatic conditions the organisms actually experience at a finer scale is problematic (Bennie *et al.*, 2008; Potter *et al.*, 2013; Woods *et al.*, 2015). Downscaling from global to microclimatic variations is needed to better investigate how ectotherms will respond to changes in their immediate environment. It is well known that local microclimate can be influenced by landscape properties (e.g. Chen *et al.*, 1999). For instance, the windbreak and antifreeze role of hedges, especially those with an embankment, has been widely studied and confirmed in agricultural landscapes. Wooded and closed areas generally have lower temperature amplitudes than open areas over a daily scale (Argent, 1992; Suggitt *et al.*, 2011), but are also colder, on average, than open areas due to the retention of cold air layers by hedges (Quénol & Beltrando, 2006). The microclimatic variation that occurs between open and closed landscapes is a very complex phenomenon that depends on many elements such as slope, hedge density, topography, and solar radiation (Quénol & Beltrando, 2006; Bennie *et al.*, 2008; Suggitt *et al.*, 2011).

In the context of conservation biology, and accepting the realities of climate change and landscape simplification, there is a need to understand whether microclimatic variation due to landscape composition affects ectotherm thermal tolerance. The existence of microclimates is widely confirmed, but the roles they play with regard to small organism ecology remain understudied (Potter *et al.*, 2013; Sunday *et al.*, 2014; Woods *et al.*, 2015). Recent field studies (e.g. Suggitt *et al.*, 2011; Pincebourde & Woods, 2012; Lawson *et al.*, 2014; Maclean *et al.*, 2015) have demonstrated the importance of focusing on microclimatic variation when assessing organisms' susceptibility to climate change. These articles, along with the present study, represent the first steps towards understanding how insects will be affected by climate change at the scale of their local environment, and whether microclimatic variations due to landscape composition could allow them to exploit more favourable temperature conditions.

There is a particular need to identify the relative roles of evolution versus behavioural and physiological plasticity in moderating the impacts of global environmental changes (Deutsch *et al.*, 2008; Sunday *et al.*, 2014). Acclimatisation (i.e. the capacity to better resist previously experienced temperatures) is an important physiological trait utilised by insects to cope with thermal stress. This is a well-known phenomenon that, along with behaviour, could be a plastic trait that commonly allows ectotherms to cope with climate change (Andrew & Terblanche, 2013;

Colinet *et al.*, 2015). Body size, which is known to influence many life history traits in insects (Chown & Gaston, 2010), could also influence their thermal tolerance. Indeed, the absolute energy demand (AED) hypothesis predicts that larger individuals should be disadvantaged in the face of stressful temperature conditions, since they expend energy proportionally faster than smaller individuals (Reim *et al.*, 2006).

This study examined the ecological relationships between the thermal tolerance of insects, microclimatic variation, and agricultural landscape composition (crop size, hedge length, and type of soil cover). In particular, this work aimed to investigate how insects could adjust their phenotypic responses to temperature variation among landscapes. To address these issues, the cold tolerance and the size of aphid parasitoids, important biological control agents, were measured across a landscape composition gradient in Brittany, France. Many studies have been devoted to these species (e.g. Thies *et al.*, 2005; Le Lann *et al.*, 2008, 2014) including investigations of their thermal tolerance (Giri *et al.*, 1982; Legrand *et al.*, 2004; Le Lann *et al.*, 2011a,b; Ismail *et al.*, 2012). Aphid parasitoids are interesting biological models for landscape microclimate studies because they closely track the distribution of their hosts, especially during the larval stage when they are immobilised inside an aphid mummy and have to withstand the climatic conditions imposed upon them.

The following hypotheses were tested: (i) in the study area, microclimate varies along the landscape gradient, with temperatures in the closed landscape being on average colder but less variable than in the open landscape; (ii) if the first hypothesis is confirmed, the parasitoid thermal tolerance should be landscape dependent, with parasitoids from open, more stressful areas being more cold tolerant; (iii) due to their seasonal acclimatisation capacities, and because exposure to unfavourable cold temperatures are less frequent in spring months, parasitoids should be more cold resistant in winter than in spring; (iv) parasitoids are smaller in open areas than in closed areas; and (v) parasitoids are smaller in winter than in spring due to temperature differences between landscapes and seasons (following the AED hypothesis).

Materials and methods

Landscape gradient determination

Parasitic wasps were monitored in winter wheat fields in northern Brittany (France). Sampling took place in the vicinity of the Long Term Ecological Research Zone *Atelier Armorique* (osur.univ-rennes1.fr/za-armorique, 48°36'N, 1°32'W) along a hedgerow network landscape gradient, ranging from closed landscapes to more open landscapes. To select the fields, 16 areas (300 m radius) containing at least one wheat field were characterised with respect to the following three parameters: total hedge

length (m), average field size (hectares), and percentage grassland in the buffer zone. The land-cover over the past 5 years (2009–2013) was characterised, and only plots with annual cultures (maize or cereals) during the last 5 years were considered. This restriction was imposed in order to limit any potential effects of grasslands on arthropod communities. One wheat field was randomly selected from each area, and each area was then identified as belonging to one of three landscape units: (i) closed landscape (five fields) – high grassland density (>45%), many hedgerows (>3200 m) and small field sizes (<0.93 ha); (ii) intermediate landscape (six fields); (iii) open landscape (five fields) – low grassland density (<20%), few hedgerows (<550 m) and larger fields (>2.70 ha). See supplementary material for a map of the study area (Appendix S1), precise locations of the fields (Appendix S2), correlations between landscape variables (Appendix S3) and extreme values for the landscape gradient (Appendix S4). All of the selected fields were separated from one another by at least 600 m. Although all fields were prospected, parasitoids were only found in 12 fields (four per landscape type). All of the selected fields were managed with similar conventional agriculture practices. Preliminary results revealed no significant differences in insect thermal tolerance within landscape types. Accordingly, data from plots belonging to the same type of landscape were pooled in subsequent analyses.

Sampling sessions and meteorological records

To evaluate potential differences in insect thermal tolerance between seasons, two rounds of sampling were conducted: one in late winter (13 January–7 March 2014) and one in early spring (24 March–15 May 2014). Over the course of both sampling periods, local meteorological data were recorded using BWS200 weather stations (Campbell Scientific, Antony, France) to characterise climate differences between seasons and among landscapes. One weather station per landscape type was used, with each recording air temperature, relative humidity (using CS215 probes accurate to ± 0.3 °C and $\pm 2\%$ respectively), and wind speed (using Wind Sentry anemometer accurate to ± 0.5 m s⁻¹) once an hour during the entire sampling period. Each station had a roof and was placed 1 m above the ground. Each station was placed at least 15 m from the hedge on flat ground.

Study species

Aphidius parasitic wasps (Hymenoptera: Braconidae: Aphidiinae) are endoparasitoids of cereal aphids. These aphids (*Sitobion avenae* Fabricius, *Rhopalosiphum padi* L., and *Metopolophium dirhodum* Walker) are present during the winter in Brittany, resulting in increased crop damage at the onset of spring (Le Ralec *et al.*, 2010). Parasitoids are present throughout the year in Brittany with a succes-

sion of species between seasons (Krespi *et al.*, 1994). In this study, *Aphidius* parasitoids were included in analyses, while secondary parasitoids were excluded due to their rarity in the fields early in the season. A total of four species were found during winter (*Aphidius rhopalosiphii* De Stefani-Perez, *Aphidius matricariae* Haliday, *Aphidius avenae* Haliday, and *Aphidius ervi* Haliday) and three species in spring (*A. rhopalosiphii*, *A. Avenae*, and *A. ervi*).

Sampling methods

Parasitoids were sampled by collecting aphid mummies (i.e. parasitised aphids containing a parasitoid pupa). To assess the diversity and abundance of mummies, fields were visited twice a week during each sampling period. Sampling occurred during a 1-h period over a surface of at least 1000 m², with field borders excluded to avoid margin effects. Mummies of the three major species of cereal aphids described above were sampled (every mummy found was collected). The largest living aphids (larval stages L4 and above) were also sampled and kept in microcages to wait for possible mummification. Each microcage consisted of a plastic tube (5 cm diameter) in which wheat [*Triticum aestivum* L. v. *Saturnus* (Poaceae)] grew on a vermiculite substrate. The microcages were checked daily for 10 days and newly formed mummies were conserved. Each mummy was placed individually in a gelatin capsule (Capsule T1; Capsugel, Illkirch-Grattenstaden, France), and subsequent parasitoid emergence was verified twice per day. Aphids, mummies, and parasitoids were maintained in the laboratory after sampling at 20 ± 1 °C, $70 \pm 10\%$ relative humidity, and a photoperiod of L16:D8. The sex of parasitoids was determined, with individuals identified according to their collection site. Following thermal tolerance experiments (see below), parasitoids were freeze-killed at -20 °C and identified using an optical microscope and the identification key developed by Hullé *et al.* (2006). Host aphid species were also identified for each parasitoid using a binocular microscope (Zeiss, Marly-le-Roi, France). This enabled the determination of the composition of the parasitoid guild and the creation of quantitative food webs for both seasons. Comparisons of thermal tolerance and size between winter and spring-collected parasitoids were only made for *A. rhopalosiphii* because it was the only species present in large numbers during both seasons.

Cold tolerance measurement

Non-lethal thermal thresholds are states of narcosis, offering a means of estimating the cold tolerance of individuals, which can have important effects on insect fitness (Le Lann *et al.*, 2011a; Alford *et al.*, 2012a). Insect physiological thermal tolerance is defined as the temperature range between the two thresholds of minimum critical temperature (CT_{min}) and maximum temperature (CT_{max})

beyond which survival is impossible (Bale, 1996). Indeed, survival is of little benefit if locomotion is compromised, leading to death through an inability to feed or to escape predators or parasites (Alford *et al.*, 2012a,b). Here, we only focus on the CT_{min} threshold. Indeed, in temperate climates, insect fitness is expected to increase as temperature rises (Deutsch *et al.*, 2008), while sudden cold spells in winter could have a major impact on parasitoid populations and could decrease the effectiveness of pest control in late winter and early spring.

To measure parasitoid cold tolerance (CT_{min}), a double-walled glass column based on the model of Huey *et al.* (1992), and improved by Powell and Bale (2006), was used. A cryostat (CryoBath®, VWR, Fontenay-sous-Bois, France) was used to cool the tube. There was no temperature difference between the top and the bottom of the tube. Temperature was controlled using a type T thermocouple probe (VWR, Fontenay-sous-Bois, France) (accurate to 0.01 °C) connected to a digital display. A cooling rate of 0.75 °C min⁻¹ was chosen to avoid inducing a rapid cold hardening response in the test insects (Powell & Bale, 2006). Relative humidity in the tube, which could not be directly controlled, was $35 \pm 10\%$. Each of the 272 parasitoids tested was individually placed in a tube. An individual's CT_{min} was considered to have been reached when the parasitoid could no longer cling to the wall of the vertical column, and dropped from the tube wall (Le Lann *et al.*, 2011a). After CT_{min} measurements, individuals were placed individually in Eppendorf® tubes (Eppendorf, Montesson, France) and stored in the freezer. In order to avoid any confounding factors (temperature, atmospheric pressure, and solar radiation in the laboratory), individuals originating from different field types were tested on each day of the experiments.

Size measurements

The size of parasitoids was measured after thermal tolerance experiments. The average length of each parasitoid's two hind tibia (a good proxy for parasitoid size) was measured and averaged. Images were captured with a AxioCam ERc5s® HD camera (Zeiss, Marly-le-Roi, France) mounted on a ×9 binocular magnifier, and measurements (precise to 0.01 mm) were performed with the Image J® Software (v. 1.48, NIH, Bethesda, MD, USA).

Statistical analyses

Statistical analyses were performed using R Software (R Development Core Team, 2013). Statistical analyses were carried out with a statistical error risk of 5%. Statistical differences in meteorological data among landscape types were tested using a standard repeated-measures ANOVA. The meteorological variable in question was considered as a fixed effect, and recording time was included as a random effect. Tukey HSD *post hoc* tests were used to detect

pairwise differences among landscape types for each season. Homogeneity of species distributions among landscapes was tested using a chi-square test of homogeneity for each season. A generalised linear model (GLM) was fitted to parasitoid CT_{min} data in both seasons, with landscape type, parasitoid species, sex, size, aphid host, parasitoid species × landscape interaction, and parasitoid species × aphid host species interaction as explanatory variables. The effect of these explanatory variables was tested with the 'ANOVA' function from the 'car' package, which performs an analysis of variance (type II) with likelihood-ratio (LR) tests, using a chi-square statistic (Fox & Weisberg, 2011). Tukey HSD *post hoc* tests for linear models were then performed to test for differences within categories. CT_{min} differences between seasons were tested using a Mann–Whitney test. Kruskal–Wallis tests were used to test if the landscape had an effect on the size of parasitoid individuals in each season. For *A. rhopalosiphii*, two GLMs were fitted and tested as described above. The CT_{min} response of parasitoids to season × sex and season × size interactions and of their size to a season × sex interaction was tested. Differences within seasons were then evaluated with Mann–Whitney tests.

Results

Meteorological records

For both seasons, closed landscapes were on average significantly colder than open landscapes (Table 1). With respect to its amplitude and standard deviation, temperature varied less in closed landscapes than in intermediate and open landscapes. In winter, closed and intermediate landscapes had a higher relative humidity than open landscapes. Closed landscapes had a lower wind speed than intermediate and open landscapes. The weather in winter was on average colder, with greater wind exposure and humidity than in spring for all landscape types. There was more variation among landscapes in winter than in spring for all of the meteorological variables measured. The occurrence of days which contained at least one temperature event under zero were for closed, intermediate, and open landscapes, respectively, 1, 2, and 4 in winter and 0, 0, and 4 in spring.

Parasitoid communities

The parasitoid community (Fig. 1) was dominated by *A. rhopalosiphii* in winter and spring. The second most abundant species was *A. matricariae* in winter, although this species was completely absent in spring. *A. avenae* was the second most abundant species in spring. The aphid community was dominated by *R. padi* in winter and *M. dirhodum* in spring. In winter, *A. matricariae* and *A. ervi* were never reared from *M. dirhodum*. In spring, parasitoids were reared from different host species in

Table 1. Daily average meteorological data ($\pm$ SE) in the study area (Brittany, France) in winter (13th January–7th March) and spring (24th March–15th May) for different types of landscape.

Season	Landscape type	Air temperature ($^{\circ}$ C)	Min Max temperature ($^{\circ}$ C)	Relative humidity (%)	Wind speed (m.s $^{-1}$)
Landscape comparison					
Winter	Closed	6.58 $\pm$ 0.07 ^a	−0.32 14.61	90.82 $\pm$ 0.25 ^a	2.18 $\pm$ 0.04 ^a
	Intermediate	7.23 $\pm$ 0.08 ^b	−0.53 23.37	90.18 $\pm$ 0.25 ^a	3.39 $\pm$ 0.06 ^b
	Open	8.39 $\pm$ 0.16 ^c	−2.07 24.91	85.82 $\pm$ 0.54 ^b	2.67 $\pm$ 0.07 ^c
	ANOVA (d.f. = 2)	$F = 67.01, P < 0.001$		$F = 67.8, P < 0.001$	$F = 148.2, P < 0.001$
Spring	Closed	10.78 $\pm$ 0.10 ^a	0.51 20.10	83.63 $\pm$ 0.40	1.84 $\pm$ 0.03 ^a
	Intermediate	11.12 $\pm$ 0.11 ^{a,b}	0.08 20.89	84.46 $\pm$ 0.38	1.91 $\pm$ 0.04 ^a
	Open	11.49 $\pm$ 0.12 ^b	−1.02 21.78	83.66 $\pm$ 0.43	2.71 $\pm$ 0.05 ^b
	ANOVA (d.f. = 2)	$F = 10.1, P < 0.001$		n.s.	$F = 130.4, P < 0.001$
Seasonal comparison					
Winter	All	7.12 $\pm$ 0.05	−2.07 24.91	89.70 $\pm$ 0.18	2.77 $\pm$ 0.03
Spring	All	11.13 $\pm$ 0.06	−1.02 21.78	83.91 $\pm$ 0.23	2.16 $\pm$ 0.03

Results of repeated-measures ANOVA comparing meteorological factors among landscapes are shown. Superscript letters (a, b, c) denote significant differences among landscapes for each meteorological factor within each season.

n.s., not significant.

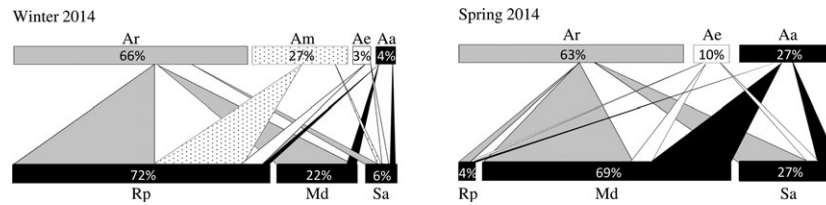


Fig. 1. Quantitative food webs of the parasitoid and aphid community composition in winter and spring 2014. Ar (*Aphidius rhopalosiphii*), Am (*Aphidius matricariae*), Ae (*Aphidius ervi*), Aa (*Aphidius avenae*), Rp (*Rhopalosiphum padi*), Md (*Metopolophium dirhodum*), Sa (*Sitobion avenae*). Upper bars represent parasitoid relative abundance and lower bars represent aphid relative abundance for each species. The thickness of the arrows between two bars is proportional to the relative number of trophic interactions between species (e.g. in winter 56% of the parasitoids emerged from *R. padi* mummies were *A. rhopalosiphii*). The figures do not include individuals that could not be identified. The total number of individuals are $n = 127$ and 141 for parasitoids and $n = 141$ and 142 for aphids in winter and spring, respectively.

accordance with the hosts' relative abundance. The species were homogeneously distributed across all landscape types in both winter (chi-square test, $\chi^2 = 7.88$, d.f. = 6, $P = 0.25$) and spring ($\chi^2 = 1.32$, d.f. = 4, $P = 0.86$).

Thermal tolerance

All parasitoid species. Figure 2 shows the data for all species (pooled) for both seasons. Statistical information is provided in Table 2. For all species and across all landscapes, the winter parasitoid community was significantly more cold resistant than the spring community (-0.64 ± 0.07 $^{\circ}$ C in winter and -0.44 ± 0.05 $^{\circ}$ C in spring, Mann–Whitney test, $W = 10273$, $P < 0.05$).

In winter, there was a significant effect of landscape type on CT_{min} . Individuals from open environments were significantly more cold resistant than those from intermediate (Tukey HSD, $z = -2.48$, $P < 0.05$) and closed ($z = -3.53$, $P < 0.01$) environments. There was a marginally non-significant difference between individuals from intermediate and closed environments ($z = -2.22$, $P = 0.06$). A similar but non-significant trend was

observed for spring populations. In spring, a significant effect of host species was found; parasitoids reared from *M. dirhodum* had significantly lower CT_{min} than those reared from *S. avenae* (-0.51 ± 0.06 $^{\circ}$ C, $n = 98$ and -0.20 ± 0.09 $^{\circ}$ C, $n = 39$, respectively, Tukey HSD, $z = 2.76$, $P < 0.05$). There were no differences between the CT_{min} of parasitoids reared from *R. padi* compared with those reared from other species. There were no significant differences in CT_{min} among species or between sexes, neither in winter nor in spring. There was no effect of body size on CT_{min} for either winter- or spring-collected parasitoids, and parasitoid size was not influenced by the landscape gradient in winter (Kruskal–Wallis test, $\chi^2 = 3.03$, d.f. = 2, $P = 0.22$) or spring ($\chi^2 = 0.13$, d.f. = 2, $P = 0.94$). There were no interaction effects among landscape gradient, parasitoid species, or aphid host species affecting the CT_{min} of parasitoids in winter or spring.

For A. rhopalosiphii only. Winter-sampled individuals from all types of landscape were more resistant to cold than those sampled in the spring (respectively -0.66 ± 0.08 $^{\circ}$ C and -0.45 ± 0.04 $^{\circ}$ C, ANOVA, LR = 3.32, d.f. = 1, $P = 0.05$). There was no difference in CT_{min}

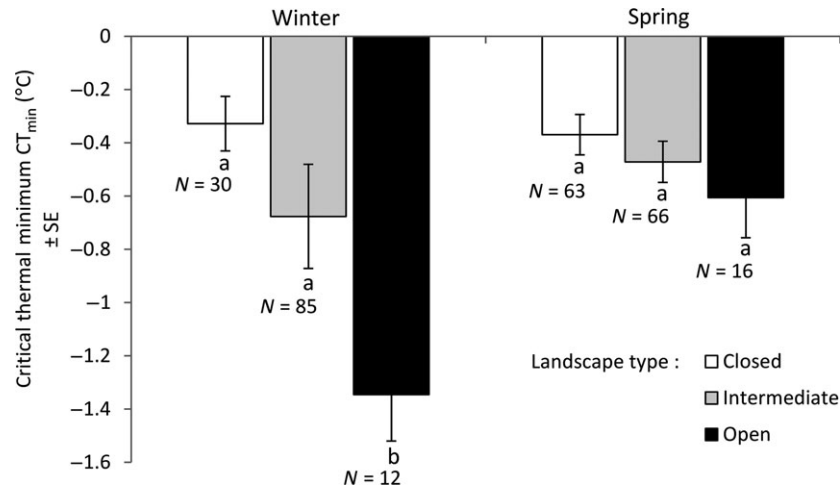


Fig. 2. Average critical thermal minimum of parasitoids (all species) in winter and spring depending on the type of landscape. $N = 127$ and 145 in winter and spring, respectively. Lower-case letters show significant differences between landscapes for each season. For inter-seasonal comparisons refer to the text.

Table 2. Factors influencing parasitoid CT_{min} .

Factor/season	Winter			Spring		
	LR (χ^2)	d.f.	$P > \chi^2$	LR (χ^2)	d.f.	$P > \chi^2$
Landscape	10.16	2	<0.01**	3.43	2	0.18
Parasitoid species	0.99	3	0.80	0.62	2	0.73
Landscape:parasitoid species	0.56	2	0.76	1.69	3	0.64
Sex	0.53	1	0.47	0.39	1	0.53
Host species	1.17	2	0.42	7.26	2	<0.05*
Parasitoid species:host species	2.16	4	0.71	0.31	4	0.98
Parasitoid size	1.96	1	0.16	0.48	1	0.49

Test statistics from generalised linear models are shown for winter ($N = 127$ individuals, 72 females and 45 males) and spring ($N = 142$ individuals, 80 females and 62 males). There were large differences in community assembly between seasons so they had to be tested separately; see 'Results' section. The likelihood-ratio (LR) chi-square is calculated for each model using the 'car' package.

*** $P < 0.001$. ** $P < 0.01$. * $P < 0.05$.

between sexes in either season (LR = 0.001, d.f. = 1, $P = 0.93$). There was no influence of parasitoids' body size on their thermal tolerance for either sex (LR = 0.63, $P = 0.42$). Furthermore, there was no effect of the interaction between season and sex (LR = 0.56, d.f. = 1, $P = 0.46$) or between season and size (LR = 0.75, d.f. = 1, $P = 0.39$) on parasitoids' CT_{min} (Fig. 3a). Winter-collected *A. rhopalosiphi* individuals were smaller than spring individuals (respectively and for both sexes, 0.50 ± 0.01 mm and 0.58 ± 0.01 mm, LR = 57.84, d.f. = 1, $P < 0.001$). There was no effect of the interaction between season and sex on parasitoid body size (LR = 0.65, d.f. = 1, $P = 0.42$). In both seasons, size differed significantly between sexes (LR = 13.03, d.f. = 1, $P < 0.001$). Females were significantly larger than males in winter (0.52 ± 0.01 mm and 0.47 ± 0.01 mm, respectively, Mann-Whitney test, $W = 1067$, $P < 0.01$) and spring (respectively, 0.58 ± 0.01 mm and 0.55 ± 0.01 mm, $W = 1098$, $P < 0.01$) (Fig. 3b).

Discussion

The results confirmed our first and second hypotheses, highlighting a landscape composition effect on different climatic factors and on the cold tolerance of parasitoids. This effect was more pronounced in winter. This study also revealed that parasitoids were smaller and more cold resistant in the winter than in spring, in accordance with our third and fifth hypotheses. Parasitoid size was not affected by the landscape gradient, which refutes our fourth hypothesis.

Landscape effects on microclimate

Between an open area and a hedged zone or forest remnant, there is generally a decrease in daytime temperatures but an increase in humidity (Argent, 1992; Quénot & Beltrando, 2006). These tendencies were confirmed by our

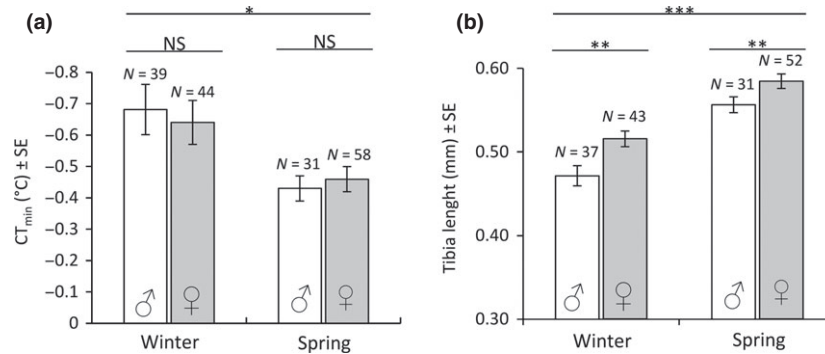


Fig. 3. Average critical minimal temperature (a) and average tibia length (b) for *A. rhopalosiphi* in winter and spring. $N = 172$ for CT_{min} measurements and 163 for size measurements. Symbols indicate parasitoids sexes males (♂) and females (♀). *** $P < 0.001$; ** $P < 0.01$; * $P < 0.05$; n.s., not significant.

measurements in winter and, to a lesser extent, in spring. Indeed, hedgerows usually limit wind speed, which acts to increase relative humidity. Additionally, cooling typically occurs along the borders of hedges and in the fields behind them. These important shelter effects limit the mixing of air layers and thus accentuate the cooling of fields surrounded by hedgerows (Quénoel & Beltrando, 2006). Another consequence of sheltered environments is that temporal variations in temperature and humidity are less pronounced than in open landscapes (Suggitt *et al.*, 2011; also confirmed by our results). In spring, temperature is less variable among landscapes than in winter, since vegetation begins to grow in the fields, and hedgerow foliage increases in density. The present study showed that open landscapes, especially in winter, present more stressful climatic conditions for ectotherms, such as wind exposure, frost, more extreme temperatures, and greater temperature variation.

Landscape effects on the cold tolerance

We demonstrated the importance of considering microhabitat variation when assessing organisms' susceptibility to climate change. Winter-sampled parasitoids from open landscapes were approximately 1 °C more resistant to cold than those from closed landscapes and 0.6 °C more resistant than individuals from intermediate landscape. The magnitude of these effects greatly decreased in spring, although a similar trend was observed. Differences in parasitoid CT_{min} along the landscape gradient may have resulted from phenotypic plasticity (acclimatisation), which is a particularly effective way for insects to cope with temperature stresses at different temporal scales (Lee & Denlinger, 2010; Colinet *et al.*, 2015). The thermal conditions encountered during the larval stage greatly influence the thermal tolerance of adults in most insects (Hoffmann *et al.*, 2013), and in open landscapes, parasitoids are less protected against cold spells. Thus, parasitoids from open landscapes could have acclimatised to sudden colder temperatures during their development,

resulting in greater adulthood cold resistance. The results of Bahrndorff *et al.* (2006) on springtails and Bauerfeind and Fischer (2014) on butterflies also support the contention that thermal extremes explain variation in thermal resistance traits better than average temperatures. Separating the effects of mean and extreme temperatures on ectotherms – a recently developing field of research in thermal biology (Easterling *et al.*, 2000; Thompson *et al.*, 2013; Colinet *et al.*, 2015) – is one of the novelties of this study. In sheltered environments, parasitoids might be better able to resist cold due to higher relative humidity, and therefore, they might have no need to develop the strong physiological resistance required of open landscape parasitoids. Indeed, as suggested by De Bach (1943), desiccation is one of the main reasons for death under cold stress conditions in Aphidiinae. Higher humidity facilitates cold resistance by diminishing the risks of desiccation (Bahrndorff *et al.*, 2006; Hoffmann *et al.*, 2013).

Winter temperatures are stressful for parasitoids, since they are often below the insects' developmental thresholds. The thermal threshold for mummy-to-adult development has been estimated at 7.2 °C for *A. rhopalosiphi*, 7.9 °C for *A. matricariae*, and 6.6 °C for *A. ervi* (Sigsgaard, 2000; Colinet & Hance, 2010). In spring, however, temperatures become warmer on average and less extreme. Thus, even if microclimatic differences along the landscape gradient persist (as it has been shown in this study), the temperatures reached are rarely or never stressful enough to make parasitoid acclimatisation necessary. For this reason, it may not be possible to reveal differences between landscapes with respect to the cold tolerance of spring-collected parasitoids. In spring, the microclimatic gradient effect could also be compensated by a smaller scale impact of vegetation within the plots. For example, it has previously been shown that vegetation cover in crops provides a stable and beneficial microhabitat for insects both in terms of temperature and wind exposure (Waterhouse, 1955). Nevertheless, as shown in this study for *A. rhopalosiphi*, parasitoid cold tolerance differs between seasons. Winter-collected individuals acclimatised

to cold temperatures are, therefore, more resistant to cold and vice versa in spring, supporting our third hypothesis.

There were no effects of sex or parasitoid species on cold tolerance in winter or spring. Parasitoids of all species emerging from *S. avenae* in spring were less cold tolerant than those emerging from *M. dirhodum*. We can thus infer bottom-up effects of host quality on parasitoid thermal tolerance. It is already known that parasitoids emerging from *S. avenae* are larger than parasitoids emerging from other aphid species (which increases their fecundity) and that parasitoids emerging from *R. padi* have a higher emergence rate (Andrade *et al.*, 2013). A trade-off may then exist between cold resistance and other life history traits that determine host choice and availability.

Size effects

In our analysis of *A. rhopalosiphi*, female parasitoids were larger than males. This trend is almost ubiquitous among parasitoid wasps (Hurlbutt, 1987) and insects in general (Chown & Gaston, 2010). There was no direct effect of size on the individuals' thermal tolerance between landscapes, although *A. rhopalosiphi* individuals were smaller in winter than in spring. If we consider that mean winter temperatures recorded are really those experienced by the parasitoids in the fields (i.e. there may have been specific locations with higher temperatures), this result does not follow the predictions of the temperature-size rule (TSR). The TSR states that insects developing at higher temperatures are generally smaller upon emergence as a result of shorter development time (Van der Have & De Jong, 1996). Our results are, however, consistent with the AED hypothesis which states that a winter should impose a selective pressure that favours smaller parasitoids. For instance, Ismail *et al.* (2012) showed that smaller individuals of the parasitoid *A. ervi* had better resistance to cold stress than larger wasps. Since parasitoid size is intimately linked to fitness-related traits (e.g. fecundity, longevity; Harvey *et al.*, 1994), there it could be a size-induced trade-off between female fertility (large individuals) and survival (reduced size) under stressful temperature conditions. These results showing an effect of temperature variations at the seasonal scale (but not at the landscape scale) confirm our fifth hypothesis but refute our fourth hypothesis concerning parasitoid size.

Conclusion

A clear impact of the landscape on the thermal tolerance of parasitoids in winter, and to a lesser extent in spring, was observed in this study. The physiological thermal tolerance of parasitoids seemed to respond to the varying weather conditions (especially extreme temperatures and relative humidity) encountered among landscapes and also among seasons. As stated in the introduction, broad scale temperature variations and predictions can be very differ-

ent from those directly experienced by insects. These data highlight the need to better understand the relationship between insect physiology and landscape structure, especially under stressful conditions. We must use fine scale studies combined with information on the microhabitat if we are to understand climate change effects on insect populations and individual physiology. Climate change effects could be exacerbated by agricultural intensification leading to more open landscapes, subjecting insects to increasing physiological stress in the future. From a biological control perspective, our results imply that it may be possible to manipulate parasitoid cold resistance using protective elements in the landscape that reduce microclimatic variations.

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Supporting Information

Additional Supporting Information may be found in the online version of this article under the DOI reference: doi: 10.1111/icad.12153:

Appendix S1. Map of the sampling zone in northern Brittany (France) with the approximate location of the 16 fields sampled.

Appendix S2. Geographic coordinates of the 16 fields sampled.

Appendix S3. Correlations between the three variables used for landscape gradient determination.

Appendix S4. Variable values for the two 300-m radius buffers (on 16 areas described) situated at the minimum and maximum extremes of the landscape gradient.

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