



Behavioural thermoregulation in *Acyrtosiphon pisum* (Homoptera: Aphididae): the effect of parasitism by *Aphidius ervi* (Hymenoptera: Braconidae)

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Abstract

The effects of parasitisation by *Aphidius ervi* on the thermoregulatory behaviour of the pea aphid *Acyrtosiphon pisum* were studied in alfalfa fields and in an experimental thermal gradient. In the field, mummies were found exclusively on the adaxial surface of the upper leaves, and aphids in the mid canopy. The adaxial surface of the upper leaves was ca. 2°C hotter than the mid-canopy. In the thermal gradient, the thermal effect (selected minus exposure temperature) was higher in magnitude in non-parasitised than in parasitised aphids; the thermal effects of both types of aphids were linearly and negatively correlated with exposure temperature (i.e. aphids showed negative thermal sensitivity). The thermal sensitivity of parasitised aphids was lower than that of non-parasitised aphids. The results are discussed in relation to hypotheses on factors governing the host-parasite relationship. © 2001 Elsevier Science Ltd. All rights reserved.

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1. Introduction

Environmental variations impose limits to the patterns of animal distribution (Andrewartha and Birch, 1954). Variation of the thermal environment is particularly relevant for ectotherms since temperature has a direct effect upon physiological and ecological processes (Reynolds, 1979; Reynolds and Casterlin, 1979; Huey and Stevenson, 1979; Watt, 1997; Zhou et al., 1997). In insects of small body size the main means of thermoregulation is behavioural (Kingsolver and Watt, 1983; Willmer and Unwin, 1981), and thermal patches of different quality are selected in order to maintain

internal temperatures favourable for physiological processes (Reynolds, 1979; Reynolds and Casterlin, 1979; Magnuson et al., 1979).

Studies of thermoregulation in insects have emphasised the temperature at which organisms tend to spend most their time (i.e. thermal preference; Reynolds and Casterlin, 1979), and the adaptations evolved towards finding such temperature in variable environments (e.g. Taylor, 1981; Kingsolver and Watt, 1983; Gilchrist, 1995). Particularly, studies about thermal ecology in aphids have described the costs and benefits of different behaviours such as walking or dropping from their host plant (e.g. Dill et al., 1990). On the other hand, studies with aphid parasitoids have demonstrated the existence of a thermal patch that maximises fitness-related activities such as locomotion performance (Gilchrist, 1996). However, although host–parasite interactions play an important role in the control of aphids in

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natural communities and agro-ecosystems (Levins, 1968; Mackahuer and Völkl, 1993), few studies have addressed the thermal ecology and behavioural thermoregulation in host–parasitoid complexes (e.g. Müller and Schmid-Hempel, 1993).

In this study, the effect of parasitisation by the parasitoid wasp *Aphidius ervi* Hal. (Hymenoptera: Braconidae) on the thermoregulatory behaviour of its host aphid *Acyrtosiphon pisum* (Harris) (Hemiptera: Aphididae), was addressed. We predict that parasitisation will alter locomotory activity, thermal sensitivity and microhabitat selection of the host. Then, the main objectives of this work were to evaluate the microhabitat and microclimate where aphids and mummies occur in the alfalfa canopy, and to evidence differences in the thermoregulatory behaviour and thermal sensitivity between parasitised and non-parasitised aphids exposed to an experimental thermal gradient. Our results are discussed in the context of hypotheses put forward to explain behavioural changes in aphids following parasitism.

2. Materials and methods

2.1. Field data

Sampling was performed in order to characterise the microhabitat (organ within the plant) and the microclimate (temperature at the microhabitat) where aphids and mummies (*A. pisum*) occur on the canopy of alfalfa (*Medicago sativa* L.). Field temperatures were recorded using a radiometer (Infratrace 801 radiometer, Kane May Ltd). Sampling was performed at two locations of central Chile (Inia-La Platina and Buin) during the austral spring of 1997, between 12:00 and 16:00 PM on sunny days, a period which included the time when the sun was at its zenith. Fields were not heavily infested by aphids, presumably due to heavy and frequent rainfalls ascribed to the El Niño Southern Oscillation phenomenon (e.g., Jaksic, 1998). A total of 65 alfalfa plants were examined for aphids and mummies during 10 min each. In 25 of them, aphids — all of them adults — were found, and in 40 of them mummies were found. Once an aphid or mummy was located, its position (upper or midcanopy) and the temperature at that site were recorded. Thermal differences between microhabitats (upper or midcanopy) were tested using Student's *t* test pooling data from both study sites.

2.2. Laboratory data

Aphids (*A. pisum*) and mummies were collected from alfalfa fields and carried to the laboratory in order to develop stock cultures. Aphids were reared on broad beans (*Vicia faba* L., cv. Banner) and used as hosts for

the parasitoid *A. ervi*, whose adults were reared on diluted honey. All stock cultures were maintained in a culture room at 20°C with a 2°C range, and 16:8 light–dark photoperiod. Non-parasitised adult aphids of similar size were withdrawn from this stock culture and used in the experiments. Each mated parasitoid female was used to parasitise 4 to 5 second-instar aphid nymphs. Parasitised aphids were placed on *V. faba* leaves during 7 days in the culture room before being transferred to the experimental thermal gradient. Two extreme conditions in aphids were addressed: non-parasitised adult aphids, and parasitised aphids with parasitoids at late developmental stages just prior to mummification (Stary, 1988).

A thermal gradient was constructed by placing a hot plate (Thermolyne, Barnstead Co. USA) as heat source below one extreme of the 5-mm thick acrylic floor of an acrylic cage (5 cm H × 5 cm W × 50 cm L). Given that parasitised aphids may show photokinetic responses (Brodeur and McNeil, 1990), light was totally excluded using a reflective surface acrylic material. Thigmokinetic responses (Brodeur and McNeil, 1990) were also standardised by lining the internal surface of the acrylic cage with a rough plastic surface (Safety Walks, 3M, USA). Surface temperature (°C) was recorded using a digital thermocouple (Digisense, Cole/Parmer Co.) which sensed areas of ca. 1 mm². The gradient was first characterised by performing temperature measurements at different distances from the extreme with the heat source. The temperature varied linearly with respect to distance from the heat source in the thermal gradient (slope of regression line = $0.197 \pm 0.003^\circ\text{C}/\text{cm}$, $t = 35.39$, $p < 0.0001$, $R^2 = 0.968$, $n = 100$). The extreme values observed were 22.0 and 32.1°C.

Twenty-nine individual trials for non-parasitised and 34 for parasitised aphids were performed in the thermal gradient inside the culture room. In each trial one aphid was placed randomly on the surface and the temperature at the site measured (initial exposure temperature). These initial locations were distributed along the entire gradient. In order to minimise aphid stress and avoid aphid desiccation, and given the low thermal inertia of aphids, each test lasted only for 5 min, after which the locomotory status (walking or resting) and the surface temperature at the point where the aphid was found (final selected temperature) were recorded. Initial exposure temperature and final selected temperature for all aphids tested fell within the 95% predicted band of the regression line describing the gradient, thus demonstrating the stability of the gradient during experimentation. Parasitised and non-parasitised aphids were subjected to similar initial exposure temperatures (ANOVA: $F[1,56] = 0.0$; $P = 0.99$). Behavioural responses displayed by parasitised aphids in the gradient were evaluated by fitting a linear logistic regression for binary data (resting or walking) by the method of maximum

likelihood (SAS Institute, 1988). The experimental points, with $p = 1$ or 0 for resting or walking, respectively, were fitted to the equation: $\text{logit}(p) = \alpha + \beta$ initial exposure temperature. The predicted probability that an aphid is resting when exposed to a given temperature, was calculated as $p = e^{\text{logit}(p)} / (1 + e^{\text{logit}(p)})$, where $\text{logit}(p)$ was estimated using the observed value of exposure temperature.

In each experimental trial, the thermal effect ($\Delta T = \text{final selected temperature} - \text{initial exposure temperature}$) for non-parasitised and parasitised aphids was calculated. Thermal sensitivity was described by a plot of the thermal effect (ΔT) as a function of initial exposure temperature. The slope of the line describes the sensitivity of aphids to environmental thermal condition (e.g. Gilchrist, 1996). Differences in the rate of change of ΔT with exposure temperature between non-parasitised and parasitised aphids were tested with an analysis of covariance (ANCOVA), which used initial exposure temperature as independent variable, ΔT as dependent variable, and aphid status as covariate. Residual analysis for normality and homogeneity of variance assumptions was performed for each test. No significant deviations from normality and homocedasticity were observed in every case.

3. Results

Aphids and mummies were found at different micro-habitats of alfalfa. Mummies occurred exclusively on the adaxial leaf surface in the upper canopy, and aphids were found only on the mid-canopy (Fisher exact P , one-tailed, $DF = 1$, $P < 0.0001$). In the mid canopy, aphids were segregated on the abaxial leaf surface ($n = 19$) and on stems ($n = 6$). The temperature experienced by mummies in the upper canopy was significantly higher than the temperature experienced by aphids in the mid canopy ($20.6 \pm 1.7^\circ\text{C}$ and $18.6 \pm 1.1^\circ\text{C}$, respectively; t -test, $n = 65$, $P < 0.001$).

Non-parasitised and parasitised aphids showed different behavioural responses to the laboratory thermal gradient. Non-parasitised aphids were always found at a site different from the one they were initially placed at. At the end of the experiment, they were observed either resting or walking. Resting behaviour occurrence increased as initial exposure temperature decreased (logistic regression: $\text{logit}(\text{resting}) = 11.199 - 0.4469$ initial exposure temperature; D.F. = 1, $1 = 0.0047$, Fig. 1A). On the other hand, parasitised aphids, whether they moved or remained immobile during the experiment, were always found resting at the end of the experiment, independent of initial exposure temperature (Fig. 1A).

Non-parasitised aphids selected a temperature which depended on the initial exposure temperature. When initially placed at the hottest side of the gradient, non-parasitised aphids tended to select temperatures lower

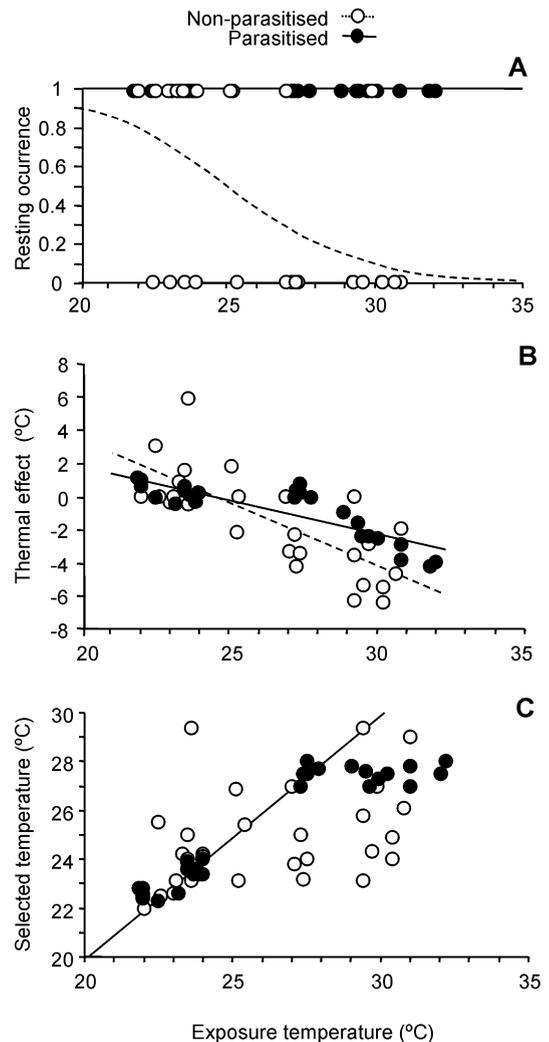


Fig. 1. Effect of thermal gradient on parasitised (solid circle and line) and non-parasitised (open circle and dashed line) aphids. (A) Logistic regressions of the occurrence of resting behaviour as a function of the exposure temperature (see equation in Section 2). (B) Thermal effect (ΔT), i.e. difference between selected and exposure temperatures, as a function of exposure temperature. The lines represent least-squares regressions for parasitised (solid line) and non-parasitised (dashed line) aphids. (C) Selected temperature as a function of exposure temperature. The line has a slope of 1, i.e. selected temperature = exposure temperature.

that the exposure temperature, i.e. they showed negative thermal effects ($\Delta T < 0$); when placed in the coldest side of the gradient, they tended to select temperatures higher than the exposure temperature, i.e. they showed positive thermal effects ($\Delta T > 0$) (Fig. 1B). On the other hand, parasitised aphids selected temperatures lower than the exposure temperature ($\Delta T < 0$) only when

initially placed in the hottest side of the gradient, and remained at temperatures not differing from the exposure temperature in all other areas (Fig. 1B). Parasitised and non parasitised aphids showed negative thermal sensitivities (slope = -0.388 ± 0.047 SE, $P = 0.0001$, $r^2 = 0.71$, and slope = -0.735 ± 0.11 SE, $P = 0.0001$, $r^2 = 0.58$, respectively), but the intensity of the responses were significantly different (ANCOVA, $F = 8.13$, $DF = 1$, $P = 0.0062$, Fig. 1B).

4. Discussion

Different predictions for the effect of parasitisation on the behaviour of aphids in a thermal gradient may be considered. On the one hand, parasitisation could affect locomotion in the host. This prediction was partially supported by the present results: while non-parasitised aphids consistently showed displacement toward temperatures different from the exposure temperature, and walking behaviour increased with increasing temperatures as reported by Dill et al. (1990), parasitised aphids showed significant displacement only when exposed to the highest temperatures in the thermal gradient. On the other hand, parasitisation may affect the reaction of the host towards the thermal environment. This prediction was supported by the results presented: parasitised aphids exploring the thermal gradient showed lower thermal sensitivity than non-parasitised aphids. The prediction is also supported if an alternative view is taken, i.e. that the response of parasitised aphids is not linear, aphids remaining immobile at temperatures lower than ca. 27°C and showing a negative thermal effect at higher temperatures. This thermal effect, however, was higher in magnitude in non-parasitised than in parasitised aphids.

Following parasitism, behavioural changes in the host have been described (reviewed by Poulin, 1995) consisting of actions by the host to kill the parasitoid by committing suicide (the so called adaptive suicide hypothesis; (McAllister and Roitberg, 1987; McAllister et al., 1990), or the manipulation of the behaviour of the host by the parasitoid to its own benefit (parasite-induced behaviour hypothesis (Brodeur and McNeil, 1989, 1992). A corollary of both hypotheses is that parasitised aphids should show a tendency to select higher temperatures than non-parasitised aphids — either to die (adaptive suicide) or to avoid hyperparasitism or predation (manipulation of the host by the parasitoid). This statement was supported by the fact that parasitised aphids showed a negative thermal sensitivity lower in magnitude than that of non-parasitised aphids (Fig. 1B), i.e. when exposed to a given hot temperature, a non-parasitised aphid selected a temperature much lower than a parasitised aphid (e.g. at a hot temperature, the magnitude of ΔT is greater for

a non-parasitised aphid). Furthermore, the site where mummies occurred in the field (i.e. adaxial leaf surface in the upper canopy) had a higher temperature than the site where non-parasitised aphids were found.

Taylor (1981) reported that the optimum temperature for development of *A. pisum* is 25°C. This temperature is consistent with that selected in the thermal gradient by non-parasitised aphids, i.e. 24.9°C (temperature at $\Delta T = 0$). Taylor (1981) also reported that maximum rate of adult development of *Aphidius rapae* occurred at ca. 27°C. Additionally, Gilchrist (1996) reported a temperature of 28°C as the optimum for locomotion activities in *A. ervi*. A temperature similar to this latter may be assumed for optimum parasitoid larval development (Levins, 1968). Interestingly, parasitised aphids actively exploring the gradient selected a temperature near 27°C. This tendency changed below 27°C (Fig. 1C): aphids tended to remain immobile, in spite of the fact that they were not at the temperature selected by exploring parasitised aphids. This effect may be ascribed to physiological constraints to movement upon parasitisation, which become evident at the lower temperatures. Since parasitised aphids tend to select a temperature most convenient to the parasitoid than to the aphid itself, the results suggest an aphid behaviour induced by the parasitoid before mummification. Furthermore, the fact that parasitised aphids exposed to the hottest temperatures of the thermal gradient — a temperature where most likely aphids would die (Taylor, 1981) — moved towards lower temperatures suggests that adaptive suicide does not operate in this system.

The results presented demonstrate the importance of thermal cues in the emergence of different behaviours upon parasitisation. Other components of the thermal environment, such as radiation, humidity, and wind speed, should be considered in future studies.

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References

- Andrewartha, H., Birch, L.C., 1954. The Distribution and Abundance of Animals. University of Chicago Press, Chicago.
- Brodeur, J., McNeil, J.N., 1989. Seasonal microhabitat selection by an endoparasitoid through adaptative modification of host behavior. *Science* 244, 226–228.

- Brodeur, J., McNeil, J.N., 1990. Overwintering microhabitat selection by an endoparasitoid (Hymenoptera: Aphidiidae): induced phototactic and thigmokinetic responses in dying host. *J. Insect Behav.* 3, 751–763.
- Brodeur, J., McNeil, J.N., 1992. Host behaviour modification by the endoparasitoid *Aphidius nigripes*: a strategy to reduce hyperparasitism. *Ecol. Entomol.* 17, 97–104.
- Dill, L.M., Frazer, A.H., Roitberg, B.D., 1990. The economics of escape behaviour in the pea aphid, *Acyrtosiphon pisum*. *Oecologia* 83, 473–478.
- Gilchrist, G.W., 1995. Specialists and generalists in environments. I. Fitness landscapes of thermal sensitivity. *Am. Nat.* 146, 252–270.
- Gilchrist, G.W., 1996. A quantitative genetic analysis of thermal sensitivity in the locomotor performance curve of *Aphidius ervi*. *Evolution* 50, 1560–1572.
- Jaksic, F.M., 1998. The multiple facets of El Niño/Southern Oscillation. *Rev. Chilena Hist. Nat.* 71, 121–131.
- Huey, R.B., Stevenson, R.D., 1979. Integrating thermal physiology and ecology of ectotherms: a discussion of approaches. *Amer. Zool.* 19, 357–366.
- Kingsolver, J.G., Watt, W.B., 1983. Thermoregulatory strategies in *Colias* butterflies: thermal stress and limits to adaptation in temporally varying environments. *Am. Nat.* 121, 32–35.
- Levins, R., 1968. *Evolution in Changing Environment*. Princeton University Press, Princeton NJ.
- Mackahuer, M., Völkl, W., 1993. Regulation of aphid populations by aphidiid wasps: does parasitoid foraging behaviour of hyperparasitism limit impact?. *Oecologia* 94, 339–350.
- Magnuson, J.J., Crowder, L.B., Medvick, P.A., 1979. Temperature as an ecological resource. *Am. Zool.* 19, 331–343.
- McAllister, M.K., Roitberg, B.D., 1987. Adaptive suicidal behaviour in pea aphids. *Nature* 328, 797–799.
- McAllister, M.K., Roitberg, B.D., Weldon, L., 1990. Adaptive suicide in pea aphids: decisions are cost sensitive. *Anim. Behav.* 40, 167–175.
- Müller, C.B., Schmid-Hempel, P., 1993. Exploitation of cold temperature as defence against parasitoids in bumblebees. *Nature* 363, 65–67.
- Poulin, R., 1995. Adaptive changes in the behavior of parasitized animals: a critical review. *Int. J. Parasitol.* 25, 1371–1383.
- Reynolds, W.W., 1979. Thermoregulation in ectotherms. *Am. Zool.* 19, 193–194.
- Reynolds, W.W., Casterlin, M.E., 1979. Behavioural thermoregulation and the “Final Referendum” paradigm. *Am. Zool.* 19, 221–224.
- SAS Institute Inc., 1988. *SAS/STAT User’s Guide*. Release 6.03 edition SAS Institute Inc. NC, USA.
- Starý, P., 1988. Natural enemies. In: Minks, A.K., Harrewijn, P. (Eds.), *Aphids: Their Biology, Natural Enemies and Control*. Elsevier, Amsterdam, pp. 171–184.
- Taylor, F., 1981. Ecology and evolution of physiological time in insects. *Am. Nat.* 117, 1–23.
- Watt, W.B., 1997. Accuracy, anecdotes, and artifacts in the study of insect thermal ecology. *Oikos* 80, 399–400.
- Willmer, P.G., Unwin, S., 1981. Field analyses of insect heat budget: reflectance, size and heating rates. *Oecologia* 50, 250–255.
- Zhou, X., Perry, J.N., Woiwod, I.P., Harrington, R., Bale, J.S., Clark, S.J., 1997. Temperature change and complex dynamics. *Oecologia* 112, 543–550.