

UNIVERSIDADE FEDERAL DO PARANÁ

MELISE CRISTINE LECHETA

**FATORES RESPONSÁVEIS PELA MANUTENÇÃO DA DISTRIBUIÇÃO  
GEOGRÁFICA E VARIAÇÃO ESPACIAL EM CARACTERÍSTICAS DA HISTÓRIA  
DE VIDA DE *Sarconesia chlorogaster* (DIPTERA, CALLIPHORIDAE)**

CURITIBA

2016

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VIDA DE *Sarconesia chlorogaster* (DIPTERA, CALLIPHORIDAE)

Tese apresentada à Coordenação do Programa de Pós-Graduação em Ciências Biológicas, Área de Concentração em Entomologia, Departamento de Zoologia da Universidade Federal do Paraná, como requisito parcial à obtenção do título de Doutor em Ciências Biológicas.

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Co-orientador: Prof. Dr. Charles W. Fox

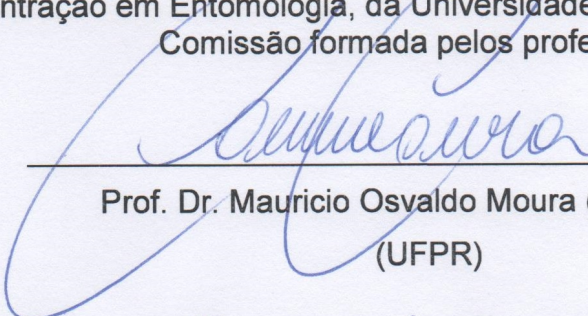
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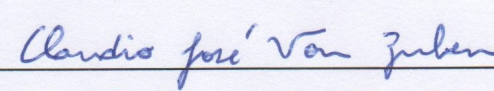
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CALLIPHORIDAE)"**

Tese aprovada como requisito parcial para obtenção do grau de Doutor em Ciências, no Programa de Pós-Graduação em Ciências Biológicas, Área de Concentração em Entomologia, da Universidade Federal do Paraná, pela Comissão formada pelos professores:



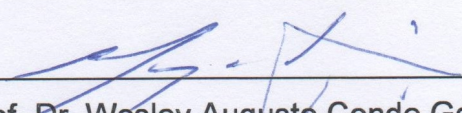
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Prof. Dr. Mauricio Osvaldo Moura (Orientador)  
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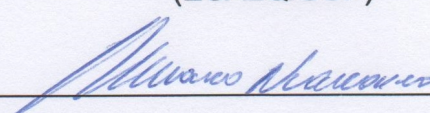
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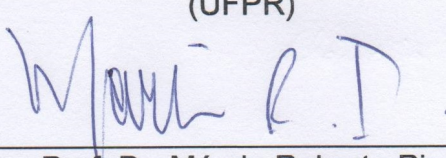
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Curitiba, 29 de fevereiro de 2016.



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Ella tenía por uno de sus mayores placeres pararse delante de la ventana en los días de lluvia y observar como el agua resbalaba por los cristales. El tintineo de las gotas contra el vidrio le hacía perder la noción del tiempo y muchas veces se imaginaba que ella misma se convertía en agua y viajaba con el viento. Algunas veces no se encontraba sola. Le parecía curioso que las moscas compartieran su afición de pararse en la ventana y mirar hacia afuera.

A ella le gustaba cantar en esos momentos, dando un concierto único para sus acompañantes. ¿Y las moscas? Las moscas la escuchaban, miraban fijamente su reflejo y juntaban sus patas, aplaudiendo. Algunas incluso bailaban.

Rubén Sarabia

## APRESENTAÇÃO

Conforme formato requerido pelo Programa de Pós-Graduação em Entomologia da Universidade Federal do Paraná, esta tese está dividida em: *Introdução*, *Objetivos* e *Capítulos* (sob a forma de artigos científicos que serão submetidos logo após a análise, correções e sugestões da banca avaliadora). Este trabalho foi desenvolvido no Laboratório de Dinâmicas Ecológicas da Universidade Federal do Paraná e The FoxLab – Evolutionary and Behavioral Ecology na University of Kentucky. A estudante recebeu bolsa de estudos concedida pelo Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) - 141043/2012-1 e bolsa período sanduíche concedida pela Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) - 99999.010306/2014-05.

## RESUMO

As espécies enfrentam diferenças nas condições abióticas ao longo da sua distribuição geográfica, sendo a temperatura um dos principais fatores que influencia diversos aspectos da vida de um organismo, especialmente em ectotérmicos. Perto da borda da distribuição geográfica a interação entre os fatores abióticos e a tolerância fisiológica pode desempenhar um papel fundamental na determinação dos limites de distribuição, já que a tolerância fisiológica determina o conjunto de condições com o qual as espécies conseguem lidar. Desse modo, a tolerância fisiológica pode restringir a distribuição geográfica da espécie. Portanto, para uma melhor compreensão de quais fatores limitam a distribuição geográfica de uma espécie é necessário conectar os limites fisiológicos com características ambientais. Nesse contexto, abordagens como a modelagem de distribuição de espécies são ferramentas úteis, já que ligam pontos conhecidos de ocorrência de uma espécie com condições climáticas. Ademais, uma maneira que os indivíduos enfrentam a variabilidade ambiental é através da plasticidade fenotípica. Portanto a variação espacial nas condições abióticas pode levar a uma diferenciação fenotípica, que pode conferir otimização local na aptidão, e resultar em diferentes estratégias entre as populações. Nesse contexto, características de história de vida podem proporcionar um melhor entendimento sobre como diferenças espaciais no ambiente são traduzidas em consequências na aptidão. *Sarconesia chlorogaster* é uma espécie que tem distribuição geográfica exclusivamente na América do Sul, restrita a regiões de clima mais frio. Estudos anteriores ressaltaram que essa espécie é sensível a temperaturas mais elevadas e estabeleceram os limites térmicos dessa espécie em 7 e 31°C. Porém, diferentes estudos sobre a biologia dessa espécie apresentaram diferenças no desenvolvimento ao longo da sua distribuição, fato que pode ser devido a diferenças no fenótipo entre as populações. Assim, o objetivo desse estudo foi determinar a resposta de *S. chlorogaster* a variáveis abióticas em diferentes escalas espaciais. Considerando que essa espécie tem sua distribuição geográfica aparentemente restrita a regiões com características climáticas específicas, nós utilizamos a abordagem de modelos de distribuição de espécies para determinar a importância do ambiente na formação de sua distribuição. Além disso, nós hipotetizamos que diferenças entre regimes climáticos ao longo de sua distribuição selecionam por mudanças nos componentes da história de vida e também nos limites térmicos entre as populações. Nós encontramos que o fator principal na formação da distribuição geográfica de *S. chlorogaster* é a resposta da aptidão ao clima, principalmente temperatura. Ademais, diferentes estratégias na história de vida são consequência ao ambiente climático em que cada população se desenvolveu. Todavia, as respostas ao choque de frio e calor não mostraram um padrão claro entre as populações. Porém, nossos resultados sugerem que o limite termal superior é mais conservado que o limite termal inferior.

Palavras-chave: características fisiológicas, modelagem de distribuição de espécies, mosca varejeira, plasticidade fenotípica, temperatura, tolerância termal, variação geográfica, variação termal.

## ABSTRACT

Species face differences in abiotic conditions along their geographic distribution and temperature is one of the main factors that influences in several aspects of an organism's life, especially for ectotherms. Near the edge of the geographic distribution, the interaction between abiotic factors and physiological tolerance plays a key role in setting up species distributional limits, since physiological tolerance determines the set of conditions a species can cope with. Thus, physiological tolerance may restrict a species' geographic distribution. Therefore, for a better understanding of which factors limit the geographic range of a species is necessary to link the physiological limits with environmental characteristics. In this context, approaches like species distribution models are useful tools, since they link known occurrence records of a species with climatic conditions. Moreover, one way organisms cope with environmental variability is through phenotypic plasticity. Thus, the spatial variation in abiotic conditions can guide to a phenotypic differentiation, which can confer a local fitness optimization, and lead to different strategies among populations. In this context, life-history traits can provide insights about how environmental spatial differences should translate into fitness consequences. *Sarconesia chlorogaster* is a species that has geographic distribution in South America, restricted to regions with colder temperatures. Previous studies showed that this species is sensitive to warmer temperatures and determined the thermal limits of this species in 7 and 31°C. However, different studies about the biology of this species showed differences in the development throughout its distribution and this can be caused by different phenotypes among populations. Thus, the aim of this study was to determine the response of *S. chlorogaster* to abiotic variables in different spatial scales. Considering that this species has its geographic distribution apparently restricted to regions with specific climatic characteristics, we used species distribution models approach to determine the importance of environment in shaping its distribution. Moreover, we hypothesized that differences among climatic regimes along its geographic distribution select for changes in life-history components and also in thermal limits among populations. We have found that the main factor shaping geographic distribution of *S. chlorogaster* is the fitness responses to climate, mainly temperature. Furthermore, different life-history strategies respond to the climatic environment where each population has evolved. Nevertheless, the responses for cold and heat shock did not show a clear pattern among populations. However, our results suggested that upper thermal tolerance is more conserved than lower thermal tolerance.

**Keywords:** blowfly, geographic variation, phenotypic plasticity, physiological traits, species distribution modeling, temperature, thermal variation, thermal tolerance.



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## INTRODUÇÃO GERAL

Dentre todas as variáveis, sejam elas bióticas ou abióticas, que podem interferir em diversos aspectos na vida de um organismo, sem dúvida a temperatura é uma das mais estudadas. Isso se deve, principalmente, ao fato da temperatura não ser somente uma propriedade da vida, mas uma propriedade da matéria (Angilletta 2009), ou seja, tudo é afetado pela temperatura. Contudo, as mesmas condições de temperatura não afetam os organismos da mesma forma. Organismos ectotérmicos são especialmente afetados pela temperatura, já que apresentam uma capacidade limitada de regular a temperatura corporal. Sendo assim, a temperatura do ambiente (e consequentemente do corpo) provavelmente é a variável mais importante que possui os maiores efeitos em todos os aspectos do comportamento e da fisiologia de um indivíduo ectotérmico (Angilletta 2009).

A temperatura do ambiente varia tanto espacial como temporalmente e afeta os organismos em diferentes escalas: na aptidão (*fitness*) e na distribuição das espécies (Fallis *et al.* 2014). A adaptação à variação térmica em grande escala (latitudinal, por exemplo) pode ser um dos fatores chaves na modulação da distribuição geográfica de uma espécie. Entretanto, variações térmicas em pequena escala podem levar a adaptação local, fazendo com que populações da mesma espécie apresentem desempenhos diferentes quando expostas ao mesmo ambiente (Richter-Boix *et al.* 2015).

A distribuição geográfica de uma espécie pode ser limitada por diversos fatores bióticos e abióticos e desmembrar o efeito dessas diferentes interações não é uma tarefa fácil. Porém, a influência das variáveis abióticas (e principalmente da temperatura) é quase sempre fundamental na determinação da distribuição geográfica das espécies, principalmente em organismos ectotérmicos. As espécies possuem diferentes limites fisiológicos, sendo que as espécies que possuem limites fisiológicos mais amplos, geralmente, também possuem uma distribuição geográfica mais ampla (Calosi *et al.* 2010). Portanto, o estudo da relação entre a distribuição geográfica da espécie com as variáveis ambientais ao longo dessa distribuição pode ser uma ferramenta valiosa para determinar os fatores que limitam a distribuição geográfica, já que cada espécie vai responder ao ambiente de forma diferente. Nesse contexto, a modelagem da distribuição de espécies (*species distribution modeling* – SDM) vem para auxiliar no conhecimento dos fatores que atuam na distribuição geográfica de um determinado organismo (Peterson *et al.* 2011). A modelagem de distribuição das espécies, em sua maioria, utiliza informações ambientais (como temperatura e precipitação) de pontos conhecidos de

ocorrência de uma espécie para extrapolar para áreas desconhecidas, predizendo a distribuição geográfica potencial. A partir desse estudo é possível determinar quais fatores são os principais atuantes na distribuição, ou ainda, sugerir quais outros fatores não levados em conta no modelo também atuam na determinação da distribuição geográfica.

Como já apontado anteriormente, as condições do ambiente (e por consequência a temperatura) atuam também em pequena escala, sendo que as espécies enfrentam variação nessas condições também ao longo da sua distribuição. Essa variação pode levar à diferenciação fenotípica entre as populações e uma otimização do valor adaptativo às condições locais. Uma das maneiras com que os organismos lidam com essa variação ao longo da sua distribuição é através da plasticidade fenotípica, produzida através da interação entre o ambiente e o processo de desenvolvimento na determinação final do fenótipo (Scheiner 1993).

Todos os organismos apresentam uma temperatura ótima de desenvolvimento e limites térmicos em torno dessa temperatura ótima nos quais são capazes de se desenvolver e deixar descendentes. Porém tanto a temperatura ótima quanto os limites térmicos de cada população são determinados principalmente pelo ambiente no qual a população se desenvolve. A variação do ambiente ao longo do ano, por exemplo, molda muitas das respostas dos indivíduos ao ambiente, porém variações de curto prazo, e no caso dos insetos variações dentro da mesma geração, também são fundamentais na determinação dos limites térmicos das populações. Ambientes mais variáveis e menos previsíveis tendem a selecionar indivíduos considerados generalistas térmicos, que são capazes de se desenvolver bem numa ampla gama de temperaturas. Da mesma forma, ambientes menos variáveis e mais sazonais tendem a selecionar indivíduos que se desenvolvem melhor em uma estreita faixa de temperatura (especialistas térmicos) (Angilletta 2009). Além disso, os organismos respondem diferentemente quando expostos a temperaturas extremas por curtos períodos de tempo (choques de frio e calor). Mesmo esses limites, principalmente o limite térmico superior, aparentemente serem mais conservados ao longo das espécies, diferenças regionais no ambiente podem provocar mudanças nas respostas a choques de temperatura entre as populações.

A espécie *Sarconesia chlorogaster* (Wiedemann, 1830) pertence à família Calliphoridae, subfamília Toxotarsinae. Essa subfamília é endêmica da região Neotropical e sua distribuição geográfica ocorre entre 4 °S e 40 °S, em regiões de clima frio e em altitudes que variam entre 0 e 4200 metros. *Sarconesia chlorogaster* tem distribuição exclusivamente

sulamericana, com registros conhecidos para a região sul do Brasil, Argentina, Uruguai, e regiões de altitude próxima a 1000 metros de Bolívia, Peru e Chile (James 1970; Dear 1979; Carvalho & Ribeiro 2000). Assim como a maioria dos califorídeos, as larvas de *S. chlorogaster* podem ter hábitos biontófagos ou necrófagos (Mariluis 1982), causando miíases obrigatórias ou facultativas, sendo importantes no contexto de saúde animal. Além disso, essa espécie também é importante na saúde pública devido à sua associação com detritos e resíduos humanos, sendo vetora de agentes causadores de doenças (Baumgartner & Greenberg 1985; Queiroz & Carvalho 1987; Maldonado & Centeno 2003; Laos *et al.* 2004). Ferreira (1978) em um estudo sobre sinantropia de dípteros muscóideos na região de Curitiba considerou *S. chlorogaster* como uma das espécies sinantrópicas mais frequentes que ocorrem nessa região. Também devido ao hábito das larvas, *S. chlorogaster* contribui para a entomologia forense fazendo parte do processo de decomposição de carcaças (Moura *et al.* 1997; Centeno *et al.* 2002; Horenstein *et al.* 2005; Souza *et al.* 2008; Horenstein *et al.* 2010), sendo reconhecida como uma das espécies mais importantes dentro da entomologia forense na América do Sul (Carvalho & Mello-Patiu 2008).

Existem poucas informações sobre a biologia e ecologia de espécies do gênero *Sarconesia*. Para *S. chlorogaster*, estudos com aspectos da biologia já foram publicados anteriormente (Greenberg & Szyska 1984; Queiroz *et al.* 1985; Bonatto 1996; Krüger *et al.* 2010, Lecheta *et al.* 2015), analisando a biologia em temperatura flutuante e constante. Esses estudos apresentaram diferenças no desenvolvimento dessa espécie ao longo da sua distribuição, fato que pode ser devido a diferentes protocolos de experimentação, mas também às diferenças no fenótipo entre as populações. Além disso, foi observado que essa espécie é capaz de se desenvolver em temperaturas baixas (~7 °C) e apresenta limite superior de temperatura em torno de 31 °C, ambos os limites térmicos sendo aparentemente mais baixos do que os comumente encontrados para espécies de califorídeos (Lecheta *et al.* 2015). Devido a esses fatores nós acreditamos que a temperatura é uma das principais variáveis que não só afeta as populações dessa espécie ao longo da distribuição (produzindo padrões locais de adaptação), mas também o fator chave que molda a sua distribuição geográfica. Portanto, esse trabalho aborda o efeito das variáveis abióticas em duas escalas diferentes: na determinação da distribuição geográfica dessa espécie (considerando todos os pontos de ocorrência conhecidos) e se diferenças térmicas regionais ao longo da distribuição dessa espécie estão gerando respostas diferentes entre as populações.

## OBJETIVOS

### Objetivo geral

Determinar a resposta de *Sarconesia chlorogaster* a variáveis abióticas em escalas espaciais diferentes.

### Objetivos específicos

1. Determinar o efeito das variáveis abióticas na formação da distribuição de *S. chlorogaster*;
2. Regimes térmicos diferentes geram respostas diferentes? O papel da temperatura na plasticidade fenotípica em características de história de vida de *S. chlorogaster*.

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## **CAPÍTULO I**

Climatic constraints shape the geographic range limits of *Sarconesia chlorogaster* (Diptera, Calliphoridae)

## INTRODUCTION

Understanding the relationship among species, their environment and the geographic patterns of distribution is a central issue in ecology (Guisan & Zimmermann 2000). A species' geographic distribution is the result of the interplay between biological and non-biological factors, such as evolutionary history, climate and biotic interactions (Gaston 1996). However, as any key biological trait, there is a limit to expand the geographic range. This limit could be produced by abiotic factors, such as physiological constraints, or imposed by biotic interactions, such as competition and predation (Sexton *et al.* 2009). Teasing apart which of these two mechanisms shapes species geographic distribution is not a simple task. Nevertheless, there is an expectation that biotic interactions would play a major role at local than at broader scales where climatic variables will be the major driver of spatial distribution (Pearson & Dawson 2003).

The influence of abiotic factors in species distributions is a largely known concept (David *et al.* 1994; Hoffmann 2003; Loh *et al.* 2008; Calosi *et al.* 2010). Near the edge of the distribution the interaction between abiotic factors and physiological tolerance play a key role in setting up species distributional limits (species margins), because physiological tolerance determines the set of conditions a species can cope with. Overall, it is expected that species with greater physiological tolerance should have wider geographic distributions (Calosi *et al.* 2010). Thus, in the absence of other factors, physiological tolerance may restrict a species geographic distribution, preventing the expansion into new areas (Kearney & Porter 2009).

For ectotherms, temperature is one of the main factors that influences several aspects of their lives, for example metabolic rate, locomotion and life-history traits (Castañeda *et al.* 2005). Organisms are unable to maximize their fitness in all environments, existing an optimum range of temperatures where they can persist. Consequently, changes in temperature directly affect the performance of the organism (Castañeda *et al.* 2005; Angilletta 2009; Schulte *et al.* 2011). When abiotic conditions become stressful and stay near to their physiological limits, fitness is reduced, affecting, among other aspects, survival and reproduction (Hoffmann 2010). Apart from biotic interactions, temperature near the edge of an organism geographic distribution usually imposes strong and/or limiting factors (Sexton *et al.* 2009). Therefore, for a better understanding of which factors limit the geographic range of a species is necessary to link the physiological limits of a species with environmental characteristics.

In this context, with the improvement of the geographic information system (GIS) and the ease to get points of occurrence of species, approaches like species distribution models (SDM) have been more applied over the recent years (Araújo & Guisan 2006; Sexton *et al.* 2009). SDM relates species distribution data (for instance, points of known occurrence) to environmental predictors at occurrence sites, assuming implicitly that the focal species maintains a viable population and that climatic variables are within the tolerance species range. When the model is fitted, points in the space that are within the multivariate climatic niche are assumed as suitable sites to the focal species. This framework has allowed a better understanding and/or prediction of the species distribution across a landscape, besides several different applications, such as conservation planning, climate change effects, species invasion and disease transmission (Elith *et al.* 2006; Elith & Leathwick 2009; Peterson *et al.* 2011).

However, many algorithms are available to model the distribution of species and, as they are built upon different assumptions, they can produce different scenarios even when using the same points of occurrence and predictor variables (Tôrres *et al.* 2012). Likewise, there are different ways to choose environmental predictors. This can be done based on species physiological limits, when this information is known, or derived from a principal component analysis (Guisan & Zimmermann 2000).

*Sarconesia chlorogaster* (Wiedemann, 1830) is a Calliphoridae (Diptera) species that has its known geographic distribution in South America (Brazil, Argentina, Uruguay, Bolivia, Peru and Chile) restricted to regions with colder temperatures (James 1970; Dear 1979; Carvalho & Ribeiro 2000). Moreover, previous studies determined the thermal limits for this species in 7 and 31°C (Lecheta *et al.* 2015). As in other calliphorid species, *S. chlorogaster* has necrophagous habitats and contributes in forensic entomology (Carvalho & Mello-Patiu 2008; Vairo *et al.* 2015; Lecheta *et al.* 2015). Furthermore, this species can be important in public health, since they can be vector for many pathogens (Baumgartner & Greenberg 1985; Queiroz & Carvalho 1987; Maldonado & Centeno 2003; Laos *et al.* 2004).

Since *S. chlorogaster* has its geographic distribution apparently restricted to regions with specific bioclimatic characteristics, we use SDM to determine the importance of environment in shaping its distribution. Specifically, we analyze the potential distribution of *S. chlorogaster* for South America, comparing five different SDM algorithms and two different sets of variables predictors. If the climatic variables are the main drivers of geographic distribution, we expect the potential distribution in South America to be close to the actual points of occurrence. These results can be used to orientate future studies about the



geographic distribution of this species, discover unknown populations, insights about the relationship between climate change and *S. chlorogaster* geographic distribution, compare the results with the ecological niche requirements of other *Sarconesia* species and survey efforts.

## **MATERIAL AND METHODS**

### **Species occurrence points**

We compiled a total of 127 occurrence records of *S. chlorogaster* obtained from different sources: 1) specimens from three zoology collections in Brazil (DZUP – Coleção Entomológica Padre Jesus Santiago Moure, Universidade Federal do Paraná; MNRJ – Museu Nacional da Universidade Federal do Rio de Janeiro; and MZUSP – Museu de Zoologia da Universidade de São Paulo) and one from Germany (ZMHB – Museum für Naturkunde der Humboldt-Universität) (20 records); 2) literature review (103 records); and 3) unpublished field records by the authors and collaborators (4 records). We used, when available, the GPS coordinates provided by the authors and for those records without this information, we used Google Earth (Google Inc. 2014) to acquire proxy geographic information from city hall coordinates (~70% of the occurrence points). Within these 127 occurrence records for *S. chlorogaster*, two records (Juan Fernández Islands and Easter Island – Chile) were excluded because WorldClim database (Hijmans *et al.* 2005) does not have climatic information available. Given the grid resolution used in this study (see below), of a total of 125 occurrences only 98 remained as unique.

### **Environmental data**

Environmental data used to produce the species distribution models included bioclimatic and topographic variables to a spatial resolution of 2.5 arc-minutes (~ 5 km) from WorldClim – Global Climate Data (Hijmans *et al.* 2005). With this database we built two sets of variables: 1) variables that were selected based on the biological knowledge of *S. chlorogaster* (biological variables hereon), and 2) variables (PCA axis) that were derived from a principal component analysis (PCA variables hereon). The biological variables were primarily selected based on the life-history response of *S. chlorogaster* at different temperatures (Lecheta *et al.* 2015). To create a subset of minimally correlated bioclimatic variables we constructed a pair-wise correlation matrix and chose those variables with coefficients of correlation <0.80, using psych package on R environment v3.2.2 (Revelle 2015; R Core Team 2015). The chosen variables were: altitude, mean diurnal range (BIO2),

temperature seasonality (BIO4), mean temperature of warmest quarter (BIO10), annual precipitation (BIO12) and precipitation seasonality (BIO15). In order to reduce dimensionality and correlation between variables, we applied a PCA approach to derive an independent bioclimatic variables set (PCA variables). A principal components analysis of the correlation matrices was performed using all 19 bioclimatic data for each *S. chlorogaster* site from WorldClim's database (Hijmans *et al.* 2005). We selected seven PCs (which accounted more than 98% of the bioclimatic variation – Table 1) and used them as our environmental variables.

### **Species distribution modeling**

Species distribution models relate field observations of species presence or abundance to environmental predictor variables in such a way that other sites with similar environmental conditions will be predicted to contain the species based on environmental similarity thresholds.

Different algorithms used in SDM have differences in data sources and statistical methods that could lead to different species potential distributions (Barry & Elith 2006; Diniz-Filho *et al.* 2009; Rocchini *et al.* 2011) and sometimes is not easy to establish which one is more adequate (Guisan & Zimmermann 2000; Diniz-Filho *et al.* 2009). One possibility to take this variation among SDM methods into account is to fit several models, compare their results and evaluate their performance (Diniz-Filho *et al.* 2009; Bedia *et al.* 2011). Therefore, to provide the most reliable potential distribution possible for *S. chlorogaster* we tested different algorithms: Envelope Score (Nix 1986; Piñero *et al.* 2007); Mahalanobis Distance (Farber & Kadmon 2003); GARP – Genetic Algorithm for Rule-set Production (with best subsets; Stockwell & Peters 1999); SVM – Support Vector Machines (Schölkopf *et al.* 2001; Tax & Duin 2004); and Maxent – Maximum Entropy (linear and quadratic functions – MaxentLQ hereon, and default – Maxent hereon; Phillips *et al.* 2006; Phillips & Dudik 2008).

Envelope Score is a quantitative version of BIOCLIM and, as well as Mahalanobis distance and SVM, is a model that uses presence data only and does not need any other information from the study area. Envelope Score is an algorithm based on climatic envelopes, one of the simplest approaches to modeling species distributions (Peterson *et al.* 2011). For each environmental variable, this algorithm finds the minimum-maximum range and the probability of presence is determined as layers within this min-max range by the total number of layers (Muñoz *et al.* 2011). Mahalanobis distance produces an ellipsoidal envelope around

the optimum ecological space, defined by the generation of a centroid for all occurrence points in the total ecological space (Nabout *et al.* 2010). This algorithm measures the distance to the mean of the observed distribution, thus, the closer a site is to the mean, the smaller the distance and the more suitable the site is (Rotenberry *et al.* 2006; Peterson *et al.* 2011). SVM is a more recent algorithm in the context of SDMs and was introduced for solving pattern recognition issues (De Marco Júnior & Siqueira 2009). This algorithm fits a hypersphere that minimally encloses known presences (Peterson *et al.* 2011) and is known for its good generalization ability (Muñoz *et al.* 2011). GARP and Maxent are more complex models that use artificial intelligence to predict the species distribution and use presence and pseudoabsence or background data (Peterson *et al.* 2011; Rangel & Loyola 2012). The background data (required for Maxent) incorporates information on environmental variation across the study area to fit the model, while pseudoabsence data (required for GARP) generates “absence” by resampling from the broader study area (Peterson *et al.* 2011). GARP develops a set of conditional rules to repeatedly find non-random correlations between observed occurrences and environmental variables (Stockwell & Peters 1999) and produces a solution that is as good as or better than the models that any component algorithm would yield (Peterson *et al.* 2011). Like SVM, Maxent has recently been applying to SDMs (Phillips *et al.* 2006). This algorithm uses the principle that the estimated distribution must agree with the information inferred from the environmental conditions at the occurrence sites, but should avoid assumptions not supported by the data (Pearson *et al.* 2007; Peterson *et al.* 2011). The final model is estimated by finding the probability distribution closest to a uniform distribution (maximum entropy) across the study area (Pearson *et al.* 2007; Phillips *et al.* 2006). The software MaxEnt was used to run Maxent (Phillips *et al.* 2006), whereas openModeller Desktop was used for the others modeling algorithms (Muñoz *et al.* 2011).

### **Evaluation of predictive performance and binary presence-absence maps of potential distribution**

To evaluate models predictive performance we divided the data set occurrence into training (70%) and testing subsets (30%), repeatedly to generate ten samples in each model. We then used a two-way ANOVA to assess the influence of the algorithm and variables set on model performance and to determinate the best variables set and modeling algorithms, using the AUC and TSS values. When we had a significant interaction term in ANOVA we broke this interaction effect into its component (main effects and levels) and then tested the separate

parts for significance. The Tukey test ( $p \leq 0.05$ ) was used to post hoc multiple comparisons using library ExpDes (Ferreira *et al.* 2013). All analyses were conducted using software R (R Core Team 2015).

We used two methods to evaluate the models predictive performance: one threshold independent (area under the receiver operating characteristic curve – AUC) and one threshold dependent (true skill statistics – TSS), suggested as an alternative to Cohen’s kappa (Allouche *et al.* 2006; Liu *et al.* 2011). AUC values vary between 0 and 1, where values equal or lower than 0.5 represent models with no predictive power (equivalent to a random model) and values greater than 0.7 indicate acceptable models (Swets 1988). TSS values vary between -1 and +1 where values equal or lower than zero indicate a performance no better than random (Allouche *et al.* 2006), while values near +1 represent perfect agreement between observed and modeled distributions. TSS takes into account both omission (known points of occurrence out the area predicted) and commission errors (including regions inside the area predicted where the species does not actually occur).

We used the threshold derived from ROC curves to generate the presence/absence distribution maps of *S. chlorogaster* for each algorithm. This threshold balances both omission and commission errors and generates smaller distribution ranges, reducing the risk of selecting sites with low suitability (Cantor *et al.* 1999; Liu *et al.* 2005).

**Table 1.** Variable coefficients of the principal components and cumulative proportion described by each principal component axis of *Sarconesia chlorogaster* populations.

Environmental variables	Principal components						
	PC1	PC2	PC3	PC4	PC5	PC6	PC7
Annual mean temperature	0.271	0.225	-0.130	0.043	-0.054	-0.018	0.019
Annual precipitation	0.262	-0.221	-0.025	-0.214	0.174	0.089	-0.095
Isothermality	0.237	0.011	0.345	-0.074	-0.232	-0.493	-0.217
Maximum temperature warmest period	0.194	0.316	-0.345	-0.017	0.134	-0.076	-0.043
Mean diurnal range	-0.179	0.219	-0.075	-0.541	0.039	-0.474	-0.320
Mean temperature coldest quarter	0.286	0.183	-0.006	0.037	-0.097	-0.028	-0.040
Mean temperature driest quarter	0.277	0.16	0.013	0.162	0.02	-0.021	-0.112
Mean temperature warmest quarter	0.232	0.262	-0.305	0.076	0.049	-0.006	0.09
Mean temperature wettest quarter	0.234	0.255	-0.234	-0.057	-0.119	-0.023	0.189
Minimum temperature coldest period	0.294	0.123	-0.007	0.165	-0.060	0.013	0.024
Precipitation coldest quarter	0.202	-0.222	0.068	0.101	0.604	-0.288	0.129
Precipitation driest period	0.143	-0.397	-0.227	-0.019	-0.130	-0.325	0.272
Precipitation driest quarter	0.154	-0.396	-0.221	-0.023	-0.099	-0.296	0.231
Precipitation seasonality	-0.039	0.328	0.409	-0.328	0.069	-0.053	0.749
Precipitation warmest quarter	0.155	-0.211	-0.199	-0.502	-0.430	0.323	0.072
Precipitation wettest period	0.267	-0.082	0.124	-0.274	0.298	0.249	-0.103
Precipitation wettest quarter	0.268	-0.090	0.113	-0.278	0.294	0.246	-0.129
Temperature annual range	-0.250	0.118	-0.315	-0.258	0.216	-0.090	-0.076
Temperature seasonality	-0.250	-0.009	-0.389	0.024	0.245	0.049	0.193
Cumulative Proportion	0.554	0.751	0.843	0.902	0.941	0.966	0.981

## RESULTS

The performance among models depended on algorithms and variables set for both AUC (significant algorithm x variables set interaction;  $F_{5,108} = 1292.2$ ,  $p < 0.01$ ) and TSS-ROC threshold (significant algorithm x variables set interaction;  $F_{5,108} = 499.8$ ,  $p < 0.01$ ).

Biological variables always performed better (AUC values) in those algorithms where biological and PCA variables were significantly different (Table 2): SVM, Maxent, Mahalanobis distance and Envelope Score. This trend was not seen when using TSS-ROC thresholds, where biological and PCA variables performance varied among algorithms (Table 3): Maxent and GARP performed better with PCA variables, while Mahalanobis distance and SVM performed better with biological variables.

Considering that: 1) biological variables had a better or equal performance than PCA variables among algorithms for AUC and in four out of six algorithms for TSS-ROC; and 2) the knowledge of biological characteristics of a species can be used for a better understanding and interpretation of the predictive distributions models (Anderson *et al.* 2003, Tognelli *et al.*

2009), we selected the biological variables (bioclimatic and topographic) as layers to model the final distributions of *S. chlorogaster*. Therefore, we evaluated the algorithms only based on biological variables.

Envelope Score was the algorithm with the lowest values independent of the threshold considered. The AUC values were always higher than 0.85 for all algorithms except for Envelope Score (Table 2). The methods with the highest AUC values (AUC = 0.91) and similar performance (statistically did not differ, Table 2) were SVM and Maxent. MaxentLQ and Mahalanobis distance also had similar performance (statistically did not differ, Table 2), although with lower AUC values than SVM and Maxent (AUC = 0.88). Regarding the TSS-ROC threshold, except for Envelope Score, all algorithms had values higher than 0.60 (Table 3). SVM had the best performance (highest TSS value 0.74, Table 3), followed by MaxentLQ (TSS = 0.69) and Maxent (TSS = 0.68), that had the same performance (Table 3).

Overall, among the algorithms tested, SVM showed the best performances for both thresholds. However, Maxent performed better in the AUC threshold, along with SVM, and the second best for TSS-ROC threshold, along with MaxentLQ. Therefore, we selected both SVM and Maxent to model the final potential distributions for *S. chlorogaster*.

In general, considering ROC threshold, *S. chlorogaster* distribution was frequent in south, southeastern and some regions of northwestern and southwestern of South America (mainly in Brazil, Uruguay, Argentina, Paraguay, Chile, Bolivia and Peru). The final distributions predicted by SVM and Maxent models showed some differences in suitable areas (Fig. 1-2). While SVM predicted a wider suitable area, Maxent predicted a narrower suitable area but with more scattered suitable patches. Both algorithms predicted south of Brazil as a suitable area, although Maxent showed a narrower distribution in south and some suitable patches in southeastern and northeastern of Brazil. Uruguay had its extension totally predicted by SVM and partially by Maxent. SVM predicted a wider area in Argentina, the country with more occurrence points, compared with Maxent, but none of the algorithms predicted the occurrence points of extreme south of this country. SVM also predicted wider areas in Paraguay and Chile than Maxent. Bolivia and Peru had almost the same area predicted by both algorithms. Moreover, Maxent predicted suitable areas in Ecuador and Colombia, countries without previous records.



**Table 2.** Evaluation of the algorithms and variables set on model performance using AUC values. Algorithms followed by asterisk (\*) differ between variables set (Tukey test  $p \leq 0.05$ ). Means followed by the same letter in the same column do not differ (Tukey test  $p \leq 0.05$ ).

Algorithm	AUC	
	Biological variables	PCA variables
SVM*	0.915a	0.586d
Maxent*	0.911a	0.896a
MaxentLQ	0.886b	0.891a
Mahalanobis distance*	0.881b	0.848b
GARP	0.852c	0.853b
Envelope score*	0.749d	0.740c

**Table 3.** Evaluation of the algorithms and variables set on model performance using TSS (ROC threshold) values. Algorithms followed by asterisk (\*) differ between variables set (Tukey test  $p \leq 0.05$ ). Means followed by the same letter in the same column do not differ (Tukey test  $p \leq 0.05$ ).

Algorithm	TSS (ROC threshold)	
	Biological variables	PCA variables
SVM*	0.742a	0.288e
Maxent*	0.684b	0.701a
MaxentLQ	0.696b	0.697a
Mahalanobis distance*	0.652c	0.582c
GARP*	0.633c	0.666b
Envelope score	0.494d	0.482d



**Figure 1.** Final distribution for *Sarconesia chlorogaster* predicted by SVM algorithm, considering the ROC threshold. The red points are the known occurrence points of *Sarconesia chlorogaster*.



**Figure 2.** Final distribution for *Sarconesia chlorogaster* predicted by Maxent algorithm, considering the ROC threshold. The red points are the known occurrence points of *Sarconesia chlorogaster*.

## DISCUSSION

Which factors shape and maintain geographic distributions are a key issue in ecology and evolution. We have found that for *S. chlorogaster* there is a close match between the potential distribution map based on SDM (SVM and Maxent) and known occurrence records. Additionally, the variable selection approach showed that physiological related variables (biological variables) performed better predicting the geographic distribution than using all the available climatic variables (PCA variables). Thus, at a larger spatial scale the main factor shaping geographic distribution of *S. chlorogaster* is associated with fitness responses to climate, mainly temperature.

Variable selection is one of the major issues for any kind of species distribution modeling (Guisan & Zimmermann 2000; Mac Nally 2000; Austin 2007). In this study, we employed physiological knowledge to select variables for our SDM when predicting the potential distribution of *S. chlorogaster*. Insects' geographic distribution should be limited by climate through physiological constraints (Kearney & Porter 2009). Therefore, knowledge about fitness response of any organism to climatic variables can be used as a variable selection approach to fit SDMs (Guisan & Zimmerman 2000). For ectothermics, temperature is one of the abiotic factors that most influences life-history traits, and consequently, their geographic distribution (David *et al.* 2003; Angilletta 2009). In this context, an organism's thermal tolerance may be one of the best characteristics to explain the latitudinal distribution of a species (Calosi *et al.* 2010).

*Sarconesia chlorogaster* has its geographic distribution closely associated with colder temperatures (James 1970; Dear 1979). The biological upper limit for the complete development of *S. chlorogaster* is about 31 °C (Lecheta *et al.* 2015), lower than usual for fly species. For example, the maximum temperature where *Drosophila suzukii* is able to develop is 42 °C (Tochen *et al.* 2014). However, *S. chlorogaster* is able to develop in lower temperatures compared to other fly species (~ 7 °C; Lecheta M.C. unp. results). For instance, *Lucilia sericata* and *Chrysomya putoria* have their minimum temperatures of development at 9.5 °C and 13.4 °C, respectively (Richards *et al.* 2009a; Gosselin *et al.* 2010). Also, the mean temperature of warmest quarter of our field records (points of occurrence) showed that *S. chlorogaster* does not occur in regions with means higher than 27.5 °C. As in the case of temperature, precipitation can influence the fitness of an ectothermic organism (Arnan & Blüthgen 2015). A study with seven blowflies' species showed that bioclimatic variables related to aridity had more influence on these species potential distributions than other

bioclimatic variables (Richards *et al.* 2009b). Furthermore, some studies reported that *S. chlorogaster* is more frequently recorded in drier regions or seasons (Baumgartner & Greenberg 1985; Horenstein *et al.* 2005; Horenstein *et al.* 2010; Aballay *et al.* 2012). Nevertheless, our data showed wider amplitude of precipitation regimes, *S. chlorogaster* occurred both in dry locations (average of 15 mm) and places with higher annual precipitation (up to 2100 mm). Another important characteristic about *S. chlorogaster* is that it has a wider altitudinal distribution (Baumgartner & Greenberg 1985). In the points of occurrence we used to fit environment models, *S. chlorogaster* occurred from 3 m up to ~ 4400 m.

The development and distribution characteristics of *S. chlorogaster* lead us to hypothesize that high temperature is a key abiotic factor that constrains the geographic distribution of this species. Therefore, we used mean temperature of warmest quarter in our SDM, along with mean diurnal range and temperature seasonality. In addition, even though *S. chlorogaster* was collected in dry and wetter places, we have chosen to include annual precipitation in our biological variables set, along with precipitation seasonality. This is because temperature and humidity are related and usually warm seasons are associated with low precipitation (Rusticucci & Penalba 2000). Furthermore, considering the wide altitudinal distribution of *S. chlorogaster*, we also considered altitude as an important topographic variable and we included it in our biological variables set. Also, because altitude and temperatures are related: usually the higher the altitude the lower the mean temperature and thermal amplitude is positively correlated with altitude (Folguera *et al.* 2008).

The use of biological variables to model the potential geographic distribution of *S. chlorogaster* (considering all algorithms and AUC and TSS-ROC thresholds) increased the algorithms' ability to predict the distributions in half the cases (6 out of 12). PCA variables had better predictions in only two algorithms using TSS-ROC threshold as a model performance. A result that also holds for Coleoptera (Corrêa 2014). Using AUC threshold, Corrêa (2014) found that when biological and PCA variables were significantly different, models fit with biological derived variables performed better. However, it did not occur using TSS-ROC or TSS-LPT thresholds (Corrêa 2014). These results suggest that adding physiological knowledge to select the variables to model potential geographic distribution may result in better predictions. Certainly, the more aspects of the life history are considered, the more accurate the models will be for organisms which geographic boundaries are mainly shaped by abiotic variables.

Of the five initial modeling algorithms, SVM and Maxent showed the best performances in predicting the occurrence of *S. chlorogaster*. However, even these two algorithms predicted slightly different potential distribution maps. The most notable difference is that Maxent showed a potential distribution more associated to the occurrence points but also with some scattered suitable patches, including countries without known records (Colombia and Ecuador) and some regions in southeastern and northeastern of Brazil. Although Colombia, Ecuador and southeastern and northeastern of Brazil are, in general, warmer, the areas predicted by Maxent are areas with higher altitudes (from ~ 750 m up to ~ 3100 m) and, probably, with colder temperatures, allowing the survivorship of *S. chlorogaster*. Despite the differences, the final distribution of SVM and Maxent are similar compared to the other models, predicting almost the same areas. Overall, our potential distributions of *S. chlorogaster* show that this species is more frequently in south and southeastern of South America, with some suitable areas in southwestern, central and northwestern. SVM and Maxent were also considered the best algorithms to predict both a bee's and its host plant species potential distributions (Silva *et al.* 2014), and Maxent the best algorithm for different species of Coleoptera (Corrêa 2014), when compared with the same algorithms used in this study.

As in the case of other taxonomic groups, *S. chlorogaster* has an uneven sample distribution along its geographic distribution, with few occurrence records in some areas. Most of occurrence records, including two new (South Brazil) are concentrated in Brazil and Argentina. Probably the forensic and medico-sanitary importance of *S. chlorogaster* and the presence of research groups in these regions are responsible for this spatial bias (for instance, Centeno *et al.* 2002; Labud *et al.* 2003; Snack & Mariluis 2004, Aballay *et al.* 2012; Horenstein & Salvo 2012). In a perfect scenario, the best model would be given when records of presence obtained from all kinds of environments were available (Araújo & Guisan 2006). This scenario is often unfeasible, however, SDM with known occurrence points available, even if the records do not represent all environments, can provide a better understanding of a species distribution, and this is not different regarding *S. chlorogaster*. With the results of this study we can suggest that the distribution of *S. chlorogaster* is shaped by physiology in response to temperature. Moreover, the result of our study can give forecasts about *S. chlorogaster* distribution in a climate change scenario and can be useful to optimize next efforts in field surveys (like Colombia, Ecuador and other countries with few occurrence points).

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## **CAPÍTULO II**

Effects of the thermal regime in life-history traits plasticity among *Sarconesia chlorogaster*  
(Diptera, Calliphoridae) populations

## INTRODUCTION

Species face different selective pressures along their geographic distribution that can be, among others, differences in abiotic conditions such as temperature and humidity (Angilletta 2009). This spatial variation in selective pressures can lead to a phenotypic differentiation in traits among populations that can confer a local fitness optimization (Via *et al.* 1995). One way organisms cope with environmental variability is through phenotypic plasticity, a developmental response to the interaction between the developmental program and the environment (Genotype x Environmental interaction; Scheiner 1993; Via *et al.* 1995). Thus, environmental variability triggers changes during development, altering the expression and connection among traits (DeWitt & Langerhans 2004), and allowing species to cope with such spatial and/or temporal environmental variability, because it places phenotypes into different selective regimes (Fordyce 2006; Angilletta 2009). However, only if the selective regime persists through time, the phenotype would move to a new peak and the plasticity would be adaptive (Ghalambor *et al.* 2007).

Life-history traits, such as developmental time and survival, can provide insights about how environmental spatial differences should translate into fitness consequences (Vergeer & Kunin 2011). As proxy for fitness measures, life-history traits can be used to compare different phenotypes among populations because of the close link between these traits and performance (Kawecki & Ebert 2004). For most ectotherms, temperature is considered a major environmental driver of life-history traits variability because of its effects on fitness (Angilletta 2009). Therefore, most studies of the effects of thermal variation have focused on life-history traits (for instance, Pétavy *et al.* 2001; Engelmann & Schlichting 2005; Ruehl & DeWitt 2005; Terblanche *et al.* 2010).

Temperature is a key factor that can cause difference in life-history strategies among populations, especially for ectotherms. Although there is a growing interest in long-term effects of temperature, such as the impact of global warming, not much is known about fine-grained or short-term thermal variation (Bozinovic *et al.* 2011). The grain of an environmental factor refers to the number of different states of that factor that an organism will encounter during its lifetime (Levins 1962; Engelmann & Schlichting 2005). If the state of the factor is constant for a short period of time (fine-grained) the organism faces several states of that factor in its lifetime, experiencing more environmental variability within lifetime (Engelmann & Schlichting 2005).

Within generations, temperature fluctuation can have important impacts on life-history traits, such as adult body size and development time, and phenotypic plasticity encompasses an important suite of mechanisms to cope with short-term variation in environmental conditions (Ragland & Kingsolver 2008; Terblanche *et al.* 2010). If an insect develops in a site with more temperature variation, the environment has a distinct effect from the environment of an insect that develops in more constant temperatures. Furthermore, the magnitude of thermal fluctuations also can have important effects on phenotypes, which may influence life-history traits (for instance, stress resistance) and, in consequence, also the fitness (Terblanche *et al.* 2010; Folguera *et al.* 2011; Paaajmans *et al.* 2013).

The fitness of any individual in a fluctuating thermal environment depends on its thermal tolerance and performance. As organisms face temporal fluctuations in temperature, the variance in temperature selects for the best strategy in each situation (a thermal generalist-specialist trade-off). When temperature fluctuates in a more predictable way, the most beneficial strategy would be a thermal specialist, i.e. an organism whose performance should be better in a narrow range of temperatures (Angilletta 2009; Gilchrist 1995). However, when temperature is less predictable, and thus more variable, a more thermal generalist strategy would evolve, i.e. a selection toward organisms that perform well over a broad range of temperatures (Angilletta 2009). The thermal tolerance of this last strategy evolves to span the range of conditions an organism is likely to experience during its lifetime (Gilchrist 1995).

Thermal gradients often change latitudinally, implying that selection should also be spatially variable (Richter-Boix *et al.* 2015). When the edge of a geographic distribution is caused by a steeper environmental gradient, it is expected that the conditions near the edge will be more stressful than at the central of the distribution. This occurs because the environmental gradients near the edge place species near of their physiological limits, reducing fitness (Sexton *et al.* 2009). Climate may have both direct and indirect effects during range expansions and contractions. Studying the responses in life-history traits among edge and central populations may help to understand the environment conditions in the edge of the distribution and how the populations deal with it.

*Sarconesia chlorogaster* (Wiedemann, 1830) (Diptera: Calliphoridae) occurs only in South America: Brazil, Argentina, Uruguay, Bolivia, Peru and Chile, restricted to regions with colder temperatures (James 1970; Dear 1979; Carvalho & Ribeiro 2000; cap I). In this study, we examined the response in life-history traits among climatic regimes of three populations from Brazil, where two of them can be considered edge populations. Traits were

measured over three temperatures in the laboratory and we compared the responses among populations. We hypothesize that differences among environments in climatic regimes will select for changes in life-history components. We expect that more variable environments (range edge populations) will select for thermal generalist strategies and more constant environments will select for a more thermal specialist strategy.

## **MATERIAL AND METHODS**

### **Characterization of the climatic regime**

To describe the climatic regime variability experienced by *S. chlorogaster* populations, we built a climatic envelope based on 41 occurrence records (with geographic coordinates provided by GPS) from Argentina, Brazil and Peru. For each population (occurrence record) we extracted nine climatic and topographic descriptors from WorldClim database ([www.worldclim.org](http://www.worldclim.org), Hijmans *et al.* 2005): altitude, mean diurnal range (BIO 2), isothermality (BIO 3), temperature seasonality (BIO 4), temperature annual range (BIO 7), mean temperature of warmest quarter (BIO 10), mean temperature of coldest quarter (BIO 11), annual precipitation (BIO 12) and precipitation seasonality (BIO 15). We chose these variables because of their effect on life-history responses of *S. chlorogaster* as well as the effect on its geographic distribution (Lecheta *et al.* 2015; cap. I). To order populations according to climatic variables and to reduce redundancy we used a principal component analysis (PCA) based on a correlation matrix with raw climatic data centered and standardized (Legendre & Legendre 1998). In order to determine the number of components to retain, the climatic descriptors in posterior analysis, we compared eigenvalues from PCA to the values given by a broken stick distribution, retaining only those components whose eigenvalue was larger than the value given by the broken stick model. In addition, we used the climatic matrix in a cluster analysis. The cluster analysis was built with a Bray-Curtis dissimilarity matrix, representing the climatic distances among populations, with an unweighted average pairs group algorithm (Legendre & Legendre 1998). As in the PCA analysis, the data was standardized before analysis.

### **Collection and maintenance of *S. chlorogaster***

We sampled three populations of *S. chlorogaster*, one in each state of south of Brazil. Populations used to start the colonies were sampled in 2013 and 2014 in Curitiba, Chapecó and Santa Maria cities (Fig. 1).

Flies were collected using a trap with rotting sardines as bait. In each location, a minimum of 20 flies (males and females) were collected. The flies were maintained from egg to adult under common-garden conditions (at 25 °C and on a light/dark cycle of 12h) until the experiments (up to 20 generations) to test for local adaptation among populations. Adults were fed with sugar, milk powder, raw ground beef and water *ad libitum* and larvae were fed with modified diet from Estrada *et al.* (2009).

### Experimental procedures

In all experiments, three incubators (122FC; Eletrolab®, São Paulo, Brazil) were used per temperature. Each incubator was adjusted to maintain controlled conditions of temperature (10, 20 or 30±1 °C), humidity (60 ± 10%), and photoperiod (12:12h). To monitor temperature and humidity, we placed thermohygrometers inside each incubator (TH-439; Equitherm®, Rio Grande do Sul, Brazil). To obtain the eggs for the experiments, raw bovine ground meat was exposed for three hours in the cages of the stock colony. Then, the eggs were immediately transferred to containers and allocated within incubators. For each population, and for each temperature, 300-420 eggs were divided into six containers. These containers were divided into the incubators to evaluate the development of egg to larvae. Each container unit was a 500 ml plastic container with 100 g of artificial diet placed inside a 1000 ml plastic container with vermiculite as substrate for pupation. The eggs/larvae were checked every 24 hours to determine the egg-larvae period, from the oviposition until third instar larvae. In order to follow the development time of the pupal stage (pupation to pupae) and adult emergence, 16 larvae per container were individually placed in test tubes containing vermiculite after they had completed feeding (wandering phase). These individuals were observed every 24 hours to determine the day of pupation, emergence of adult and mortality. To quantify adult lifespan and reproduction traits, after the emergence of these individualized pupae, five males and five females were allocated into a cage inside the incubator, with two cages per population per incubator, for each temperature. The adults were fed with sugar, milk powder and water *ad libitum* and larvae were fed with diet modified from Estrada *et al.* (2009). Raw ground beef was offered and replaced every day until all the adults were dead to check the presence and the number of eggs.

We performed a morphometric analysis of adult thorax length, with the same individuals used to quantify adult lifespan and reproduction traits, to test if adult body size differs among populations, temperature regimes and sex. Thorax length from ten males and

ten females were measured per population per incubator for each temperature. In order to test the difference in egg volume among the populations (reproductive investment), nine virgin couples from the colony stock (25 °C) for each population were isolated (one couple per cage), and five eggs were measured per couple.

### Statistical analysis

All analyses were run under the R environment v3.2.2 (R Core Team 2015). To test if there is an effect of temperature and population on egg-larval development time we fitted a linear mixed-effects model (LMM) with the rearing container as a random effect;  $Y = \text{temperature} * \text{population} + (1|\text{container})$ . The same model was fitted for pupal development time and adult lifespan, but adding sex as a factor in the analysis. To test for temperature and population effects on the first day of oviposition and total lifetime fecundity, we performed a two-way analysis of variance (ANOVA). To test for temperature and population effects on pupal survival and sex ratio we fitted a generalized linear mixed-effects model (GLMM) assuming binomial errors and container as a random effect.

To test if there is an effect of temperature, population and sex on adults' body size we performed a three-way analysis of variance (ANOVA). A one-way analysis of variance (ANOVA) was used to test the population effects on egg volume. When our analyses yielded significant results, we performed specific post hoc comparisons between pairs of populations and temperature.

Prior to fit the models we checked assumptions of normality and homoscedasticity of raw data and egg-larvae development time, first day of oviposition, total lifetime fecundity and egg volume were  $\log_{10}$  transformed. A level of  $p < 0.05$  was used to reject the null hypothesis. For the LMM and GLMM, lme4 library (Bates *et al.* 2015) was used and for the subsequent analyses, lsmeans, multcomp and doBy libraries (Hothorn *et al.* 2008; Hojsgaard *et al.* 2014; Russell 2015) were used. For PCA and cluster analyses, vegan library was used (Oksanen *et al.* 2015).



**Figure 1.** Populations sites (Brazil) used to start the colonies of *Sarconesia chlorogaster*.

## RESULTS

### Climatic envelope and the climatic regime characterization of populations

We used two methods (PCA and cluster analyses) to explore how *S. chlorogaster* fills the climatic niche space. Regarding PCA, from the nine initial components, three were selected using the broken stick method. These three components explained 49.4%, 25.9% and 21.0% of the variation respectively, giving a total variance in the original dataset of 96.3% (Table 1). Even if the PCA scatter plot did not show clear distinct groups for Argentinean populations (Fig. 2), the occurrence records of *S. chlorogaster* could be divided in four putative groups based on the PCA scatter plot and the cluster dendrogram (Fig. 3): 1) Peru – the only record for this country; 2) Argentina – records in northwestern of Argentina; 3) Argentina-Brazil – records in south and eastern of Argentina and extreme south of Brazil ; and 4) Brazil – edge records further north in Brazil. As these analyses showed (Fig. 2 and 3), the Brazilian populations occupied different positions in the climatic envelope. Both PCA scatter plot and the cluster dendrogram showed a clearly separation (in the PC2 axis) of Santa Maria from Curitiba and Chapecó – Santa Maria is in the Argentina-Brazil group, with a climatic environment more similar to some records in Argentina than records in Brazil. The scores of the variables in the second PCA axis showed that Santa Maria has greater temperature seasonality but lower isothermality (mean of all monthly diurnal temperature ranges divided by the annual temperature range) and lower altitude. In other words we can say that Santa Maria has less short-term temperature variation (more predictable environment)

than Curitiba and Chapecó relatively to its annual temperature range, reflected by isothermality. Furthermore, Santa Maria has the highest temperature means throughout the year reflected by mean temperatures of warmest (BIO 10) and coldest quarters (BIO 11), both higher in comparison to Chapecó and Curitiba.

## **Life-history traits**

### **a. Overall temperature effect**

Temperature affected nearly all traits of *S. chlorogaster*, consistent with the typical pattern in ectothermic animals. Larval development time ( $X^2_2 = 1387.0$ ,  $p < 0.001$  – Fig. 4), pupal development time ( $X^2_2 = 466.2$ ,  $p < 0.001$  – Fig. 5 and 6) and adult lifespan (except for Curitiba, see below;  $X^2_2 = 50.5$ ,  $p < 0.001$  – Fig. 7 and 8) all decreased with increasing temperature, consistent with the general pattern in ectotherms. In the case of survival and reproduction, flies generally performed the worst at 10°C and the best at 20 °C, though performance at 20 °C was often similar to 30 °C. Pupal survival ( $X^2_2 = 84.3$ ,  $p < 0.001$  – Fig. 9) was quite low at 10 °C for Curitiba and Chapecó (and zero for Santa Maria), and highest for the three populations at 20 °C. 10° C was also the worst temperature for reproduction; none of the females that survived to adult laid eggs at this temperature. Flies laid eggs earlier at 30 °C ( $F_{1,26} = 15.2$ ,  $p < 0.001$  – Fig. 10) but had greater total lifetime fecundity at 20 °C ( $F_{1,26} = 26.3$ ,  $p < 0.001$  – Fig.11). The body size was bigger at 20 °C in all three populations and similar between 10 °C and 30 °C ( $F_{2,366} = 147.0$ ,  $p < 0.001$  – Fig. 12 and 13).

### **b. Population and population-by-temperature interactions**

Even though the fitness curves differed among populations, the general response had the same direction. Performance was better (bigger values of traits) near the optimal development temperature and worse in extreme temperatures.

Flies from Curitiba and Chapecó survived to adult at the three temperatures, but flies from Santa Maria did not develop at 10 °C (none of the eggs hatched). Chapecó flies performed the best in almost all temperatures and traits; overall they had a shorter larval development time ( $X^2_2 = 7.72$ ,  $p < 0.05$  – Fig. 4), the highest pupal survival ( $X^2_2 = 32.8$ ,  $p < 0.001$  – Fig. 9), adult flies laid eggs earlier ( $F_{2,26} = 5.23$ ,  $p < 0.05$  – Fig. 10), and had greater body size ( $F_{2,366} = 16.8$ ,  $p < 0.001$  – Fig. 12 and 13). Flies from Curitiba and Chapecó produced bigger eggs than Santa Maria ( $F_{2,24} = 7.40$ ,  $p < 0.01$  – Fig. 14) and had greater total lifetime fecundity ( $F_{2,26} = 4.76$ ,  $p < 0.05$  – Fig. 11). Pupal development time ( $X^2_2 = 3.27$ ,  $p =$



0.20 – Fig. 5 and 6) did not vary among populations. Our results suggest that sex ratio might vary among populations, but the population effect in the analysis was marginally non-significant ( $X^2_2 = 5.87$ ,  $p = 0.053$ ).

The variance in adult lifespan among populations also depended on temperature and population (significant rearing temperature x population interaction;  $X^2_3 = 13.0$ ,  $p < 0.01$ ). Flies from Santa Maria had the worst lifespan ( $X^2_2 = 6.80$ ,  $p < 0.05$  – Fig. 7 and 8) at both 20 and 30 °C; flies from Curitiba, which has the coldest recorded mean temperatures, had the shortest lifespan at 10 °C ( $27.6 \pm 16.4$  days, compared to  $48.4 \pm 24.5$  days for Chapecó). The variance in total lifetime fecundity among populations also depended on temperature and population (significant rearing temperature x population interaction;  $F_{2,26} = 6.10$ ,  $p < 0.01$ ). Flies from Curitiba had the highest total lifetime fecundity of all populations when reared at 20 °C, but the lowest at 30 °C (Fig. 11). The body size also depended on population and temperature (significant rearing temperature x population interaction;  $F_{3,366} = 6.89$ ,  $p < 0.001$ ). All three populations had the greatest values of body size at 20 °C and similar lower values at 10 °C and 30 °C (Fig. 12 and 13).

### c. Sex differences

Overall, males took longer to complete pupal development than females ( $X^2_1 = 16.2$ ,  $p < 0.001$  – Fig. 5 and 6). However, this sex-difference varied with temperature. This is because temperature influenced pupal development time of females more than it influenced males ( $X^2_2 = 8.18$ ,  $p < 0.05$ ) – the difference in development time between 10 and 30 °C was ~22 days for males versus ~26 days for females. Nevertheless, there was also a significant three-way interaction (significant rearing temperature x population x sex interaction;  $X^2_3 = 7.90$ ,  $p < 0.05$ ); males and females from Chapecó at 10 °C had the greatest among-temperature variation in pupal development time.

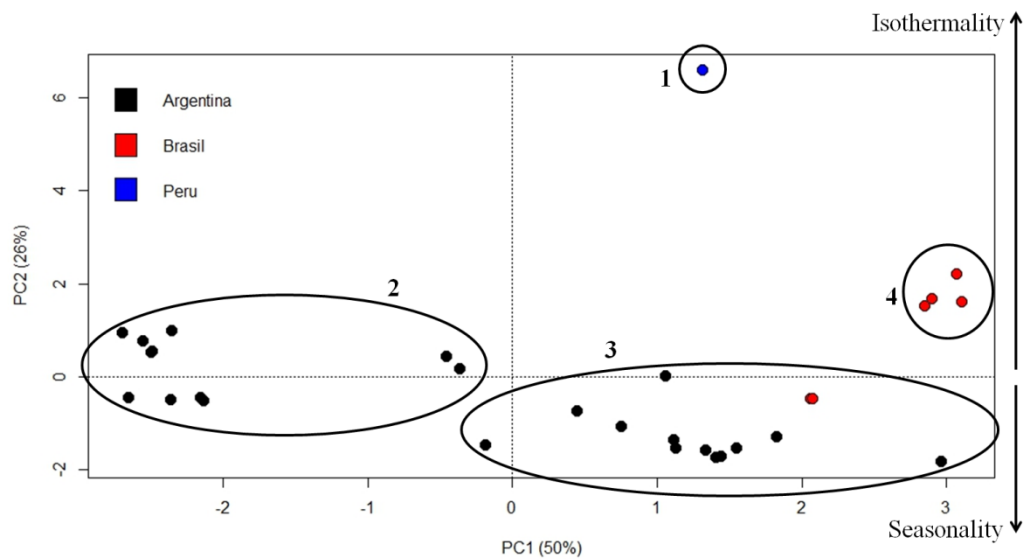
There was a complex three-way interaction (significant rearing temperature x population x sex interaction;  $X^2_2 = 8.23$ ,  $p < 0.05$ ) in which the sex-difference in lifespan depended on temperature and population; females of Chapecó and Santa Maria had shorter lifespan while females of Curitiba had longer lifespan at 20 and 30 °C (Fig. 7 and 8). In contrast to flies from Chapecó and Santa Maria that decreased their lifespan with temperature for males and females, Curitiba had longer adult lifespan at 20 °C than at 10 °C or 30 °C; flies from Curitiba also had longer adult lifespan at 30 °C for both males and females than flies from Chapecó and Santa Maria, and the shortest adult lifespan at 10 °C.

Overall, males had greater values of body size in all temperatures than females ( $F_{1,366} = 52.9$ ,  $p < 0.001$  – Fig. 12 and 13). Similarly to lifespan, males and females did not vary in body size with temperature ( $F_{2,366} = 1.29$ ,  $p = 0.28$ ). However, there was a complex three-way interaction (significant rearing temperature x population x sex interaction;  $F_{3,366} = 2.95$ ,  $p < 0.05$ ) in which the sex-difference in body size depended on temperature and population; males and females from Chapecó had greater results in all temperatures, except for females at 30 °C in which flies from Santa Maria had the greatest values (Fig. 12 and 13).

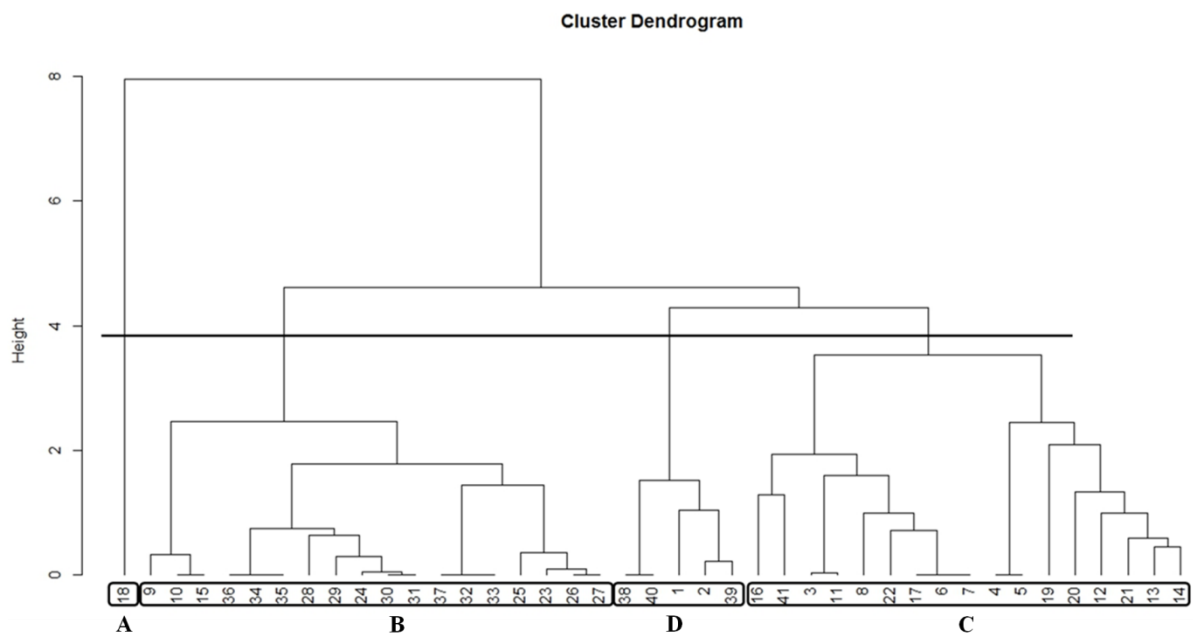
The sex ratio ( $X^2_2 = 1.82$ ,  $p = 0.40$ ) of emerging adult flies was not affected by rearing temperature.

**Table 1.** Variable coefficients of the principal components and cumulative proportion described by each principal component axis of *Sarconesia chlorogaster* populations.

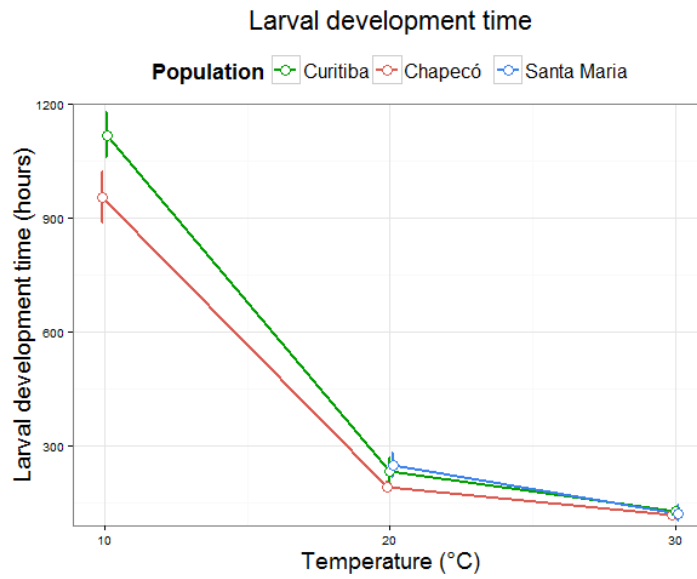
Environmental variables	Principal components		
	PC1	PC2	PC3
Altitude	-0.148	0.587	-0.167
Mean diurnal range	-0.424	0.262	0.004
Isothermality	0.144	0.604	-0.101
Temperature seasonality	-0.419	-0.293	0.044
Temperature annual range	-0.468	-0.022	0.065
Mean temperature warmest quarter	-0.287	-0.027	0.575
Mean temperature coldest quarter	0.018	0.226	0.673
Annual precipitation	0.355	0.170	0.391
Precipitation seasonality	-0.415	0.228	-0.131
Cumulative Proportion	0.493	0.753	0.963



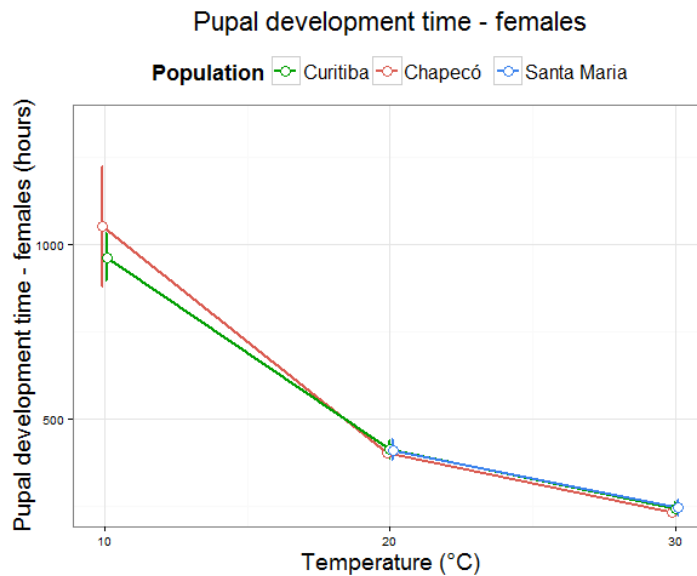
**Figure 2.** PCA scatter plot showing the first two PCs (PC1-PC2). The four groups of occurrence records are termed as 1) Peru, 2) Argentina, 3) Argentina-Brazil, and 4) Brazil.



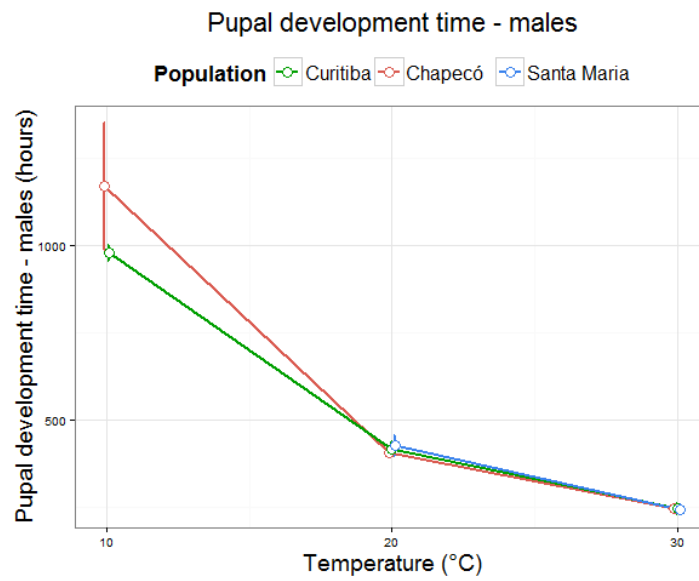
**Figure 3.** Dendrogram of cluster analysis based on Bray-Curtis dissimilarity. The four groups of occurrence records are termed as A) Peru, B) Argentina, C) Argentina-Brazil, and D) Brazil. The numbers represent the site of each occurrence record: Argentina (4-10, 12-17, 19-37, 41); Brazil (1-3, 11, 38-40); Peru (18).



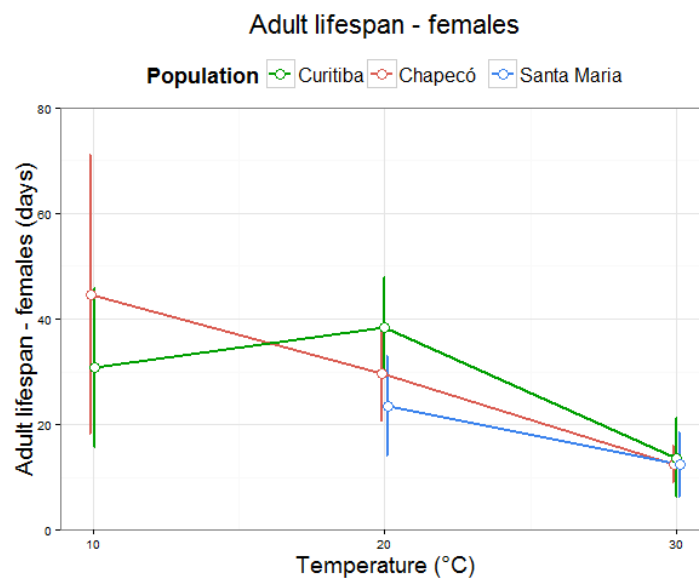
**Figure 4.** Larval development time (in hours) of populations of *Sarconesia chlorogaster* raised at three different temperatures (10, 20 and 30 °C). Standard deviations ( $\pm 1SD$ ) are included. Flies from Santa Maria did not develop at 10 °C.



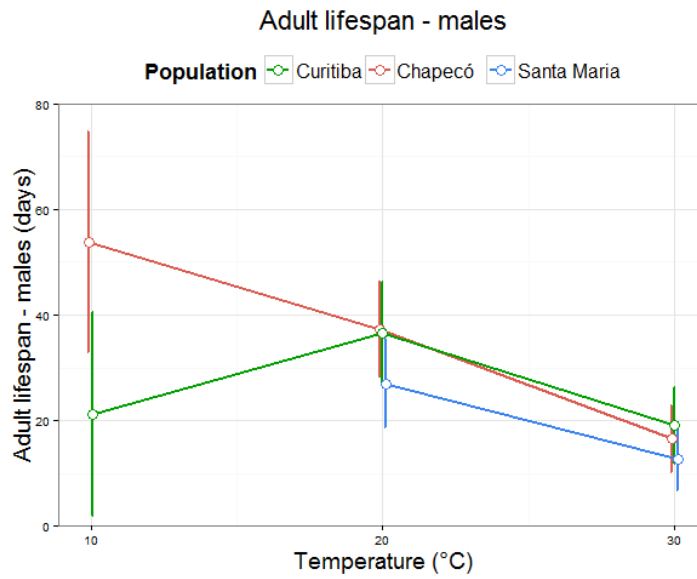
**Figure 5.** Female pupal development time (in hours) of populations of *Sarconesia chlorogaster* raised at three different temperatures (10, 20 and 30 °C). Standard deviations ( $\pm 1SD$ ) are included. Flies from Santa Maria did not develop at 10 °C.



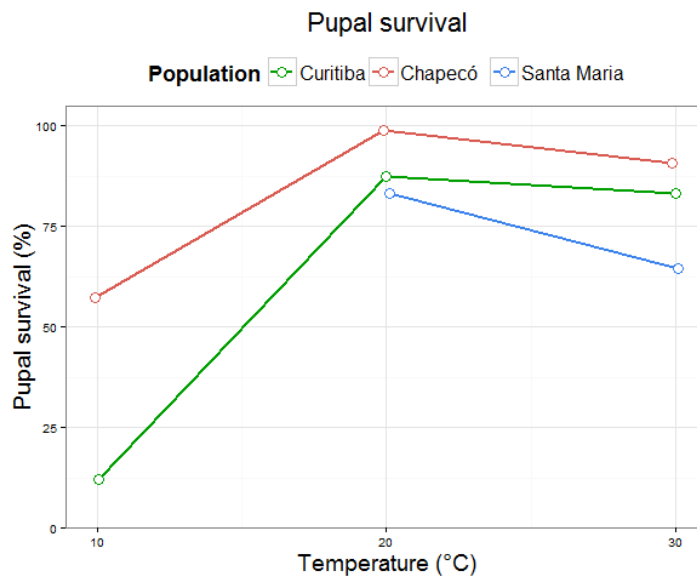
**Figure 6.** Male pupal development time (in hours) of populations of *Sarconesia chlorogaster* raised at three different temperatures (10, 20 and 30 °C). Standard deviations ( $\pm$  1SD) are included. Flies from Santa Maria did not develop at 10 °C.



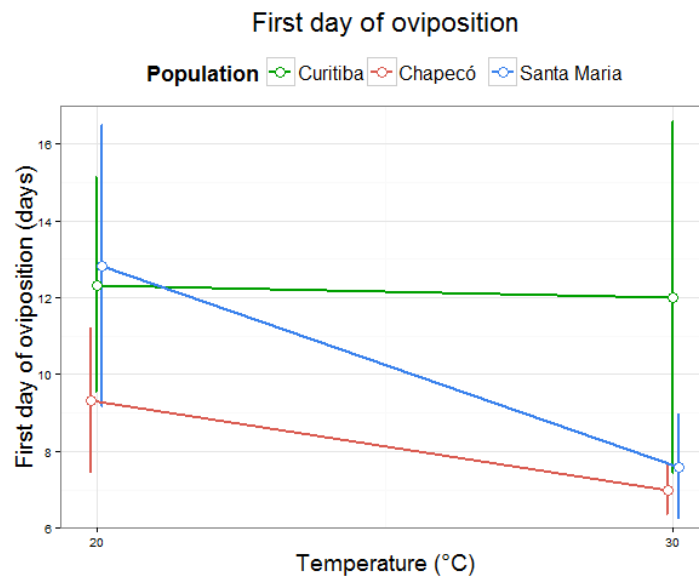
**Figure 7.** Female adult lifespan (in days) of populations of *Sarconesia chlorogaster* raised at three different temperatures (10, 20 and 30 °C). Standard deviations ( $\pm$  1SD) are included. Flies from Santa Maria did not develop at 10 °C.



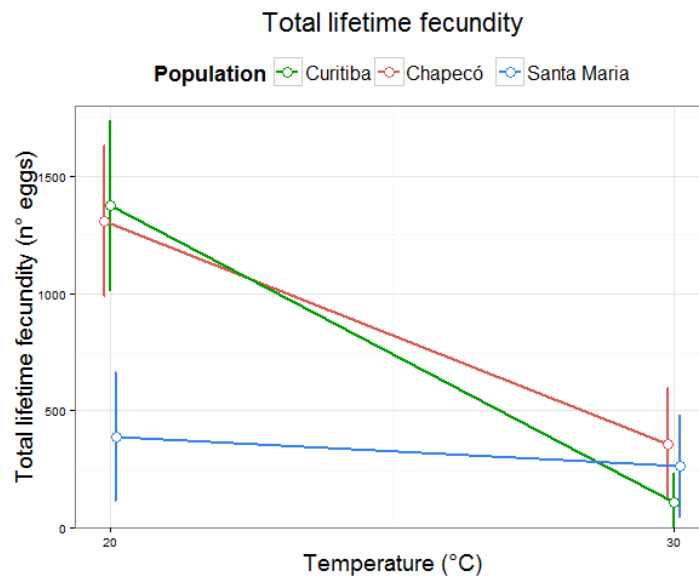
**Figure 8.** Male adult lifespan (in days) of populations of *Sarconesia chlorogaster* raised at three different temperatures (10, 20 and 30 °C). Standard deviations ( $\pm$  1SD) are included. Flies from Santa Maria did not develop at 10 °C.



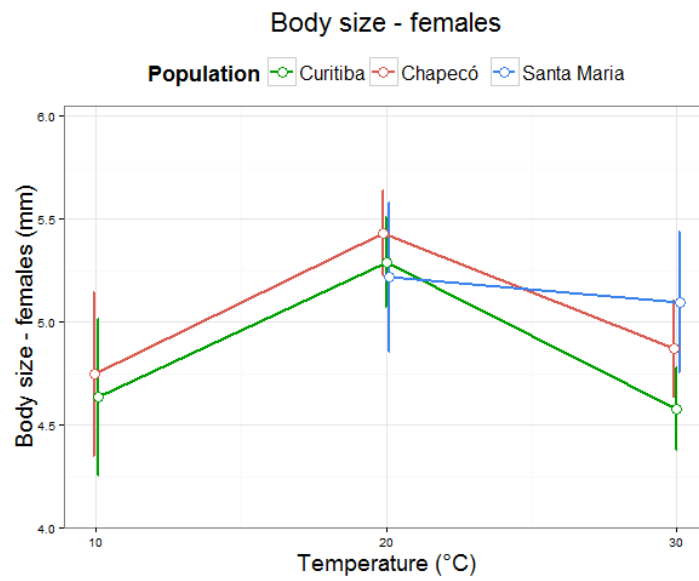
**Figure 9.** Pupal survival (percentage) of populations of *Sarconesia chlorogaster* raised at three different temperatures (10, 20 and 30 °C). Flies from Santa Maria did not develop at 10 °C.



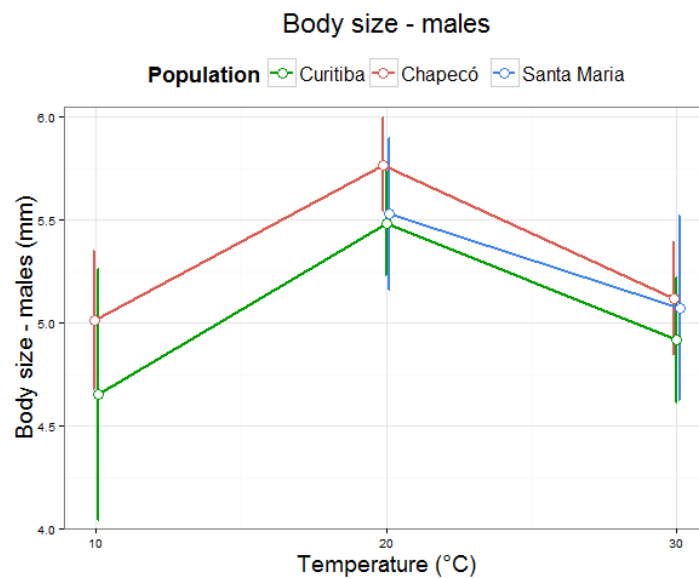
**Figure 10.** First day of oviposition of populations of *Sarconesia chlorogaster* raised at two different temperatures (20 and 30 °C). Standard deviations ( $\pm$  1SD) are included. Flies that survived at 10 °C did not lay eggs.



**Figure 11.** Total lifetime fecundity (in number of eggs laid) of populations of *Sarconesia chlorogaster* raised at two different temperatures (20 and 30 °C). Standard deviations ( $\pm$  1SD) are included. Flies that survived at 10 °C did not lay eggs.

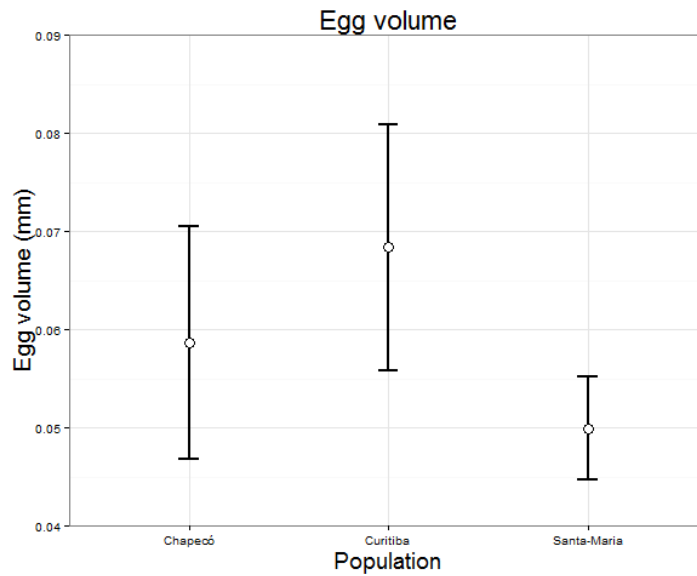


**Figure 12.** Female body size (in mm) of populations of *Sarconesia chlorogaster* raised at three different temperatures (10, 20 and 30 °C). Standard deviations ( $\pm$  1SD) are included. Flies from Santa Maria did not develop at 10 °C.



**Figure 13.** Male body size (in mm) of populations of *Sarconesia chlorogaster* raised at three different temperatures (10, 20 and 30 °C). Standard deviations ( $\pm$  1SD) are included. Flies from Santa Maria did not develop at 10 °C.





**Figure 14.** Egg volume (in mm<sup>3</sup>) of populations of *Sarconesia chlorogaster* raised at 25°C. Standard deviations ( $\pm 1SD$ ) are included.

## DISCUSSION

Temperature is one of the most important factors that can influence in traits and fitness in ectotherms organisms. Environmental temperature varies in time and space and organisms must deal with the thermal variation on a variety of ways and scales along their geographic ranges (Johnston & Bennett 1996). In this context, phenotypic plasticity plays a key role to cope with different temperatures. In this study, we suggest that different life-history strategies are a response to the climatic environment where each population has evolved.

Temperature affects time of development and survival of immature stages in the same direction in all populations, since the norms of reaction are quite similar. Overall, the responses converge with the increase of temperature and the biggest differences appear in the lowest temperature, meaning that phenotypic variance decreased with the increase of temperature. The increase of temperature from 10 to 20 °C lead flies from Chapecó to reduce adult lifespan, while flies from Curitiba increased adult lifespan. As well as time of development of immature stages, we expected lifespan to decrease with increasing temperature, since at lower temperatures there is a physiological increase in lifespan in ectotherms as metabolic rate decreases and individuals use resources slower (Brown *et al.* 2004). The non-linear response of flies from Curitiba possibly reflects their optimum developmental temperature, 20 °C (Lecheta *et al.* 2015).

Morphological reaction norms showed the same trend, with smaller flies emerging from extreme temperatures. However, there was a sex specific component for flies from Santa Maria where females had less variation in body size than males. Flies from Chapecó had bigger body sizes in all temperatures. The rule of “bigger is better” states that individuals with larger body size will tend to have greater fitness than smaller individuals (Kingsolver & Huey 2008). Larger adult body sizes may contribute positively to fecundity and survival, leading to a positive contribution to total lifetime fitness. Large adult size usually requires longer development times, what we did not observed in flies from Chapecó. However, larger eggs also can lead to larger body sizes independently of development time, what probably is guiding the larger body size in Curitiba and Chapecó. Besides, there are two rules that relate temperature of development and body size: “hotter is smaller” and Bergmann’s rule. The “hotter is smaller” rule proposes that ectotherms that develop at higher temperatures will be relatively small as adults (Kingsolver & Huey 2008). Flies from all three populations in this study reared at 30 °C were smaller than flies reared at 20 °C and overall this rule has strong empirical support in a variety of species (Kingsolver & Huey 2008). Bergmann’s rule states that individuals reared in colder temperatures tend to have larger adult body sizes however this rule cannot be generalized for ectotherms (Kingsolver & Huey 2008). As we observed in this study, flies from Chapecó and Curitiba had similar body sizes when reared at 10 °C and 30 °C but bigger ones when reared at 20 °C, therefore not following this rule. This result was also found for other species (Pétavy *et al.* 2001).

Flies from Santa Maria and Chapecó showed the same response for the first day of oviposition, although individuals from Chapecó laid eggs first in both temperatures. Flies from Curitiba did not show plasticity in this trait (slope around zero). Comparing the lifespan of females, flies from Curitiba had the longest lifespan at 20 and 30 °C and it could explain why Chapecó laid eggs earlier. This may be a response for having fewer days to lay eggs which leads to start laying eggs earlier. Flies from Santa Maria (that have the shortest female lifespan) started to lay eggs fast at 30 °C but needed almost the same number of days as flies from Curitiba to start laying eggs at 20 °C. Concerning total lifetime fecundity, individuals from Curitiba and Chapecó had the same pattern of response and flies from Santa Maria did not show plasticity. Since the adults live longer at 20 °C than at 30 °C, total lifetime fecundity was overall better at 20 °C and flies from Curitiba and Chapecó were similar and laid more eggs in this temperature. However, if we calculate the ratio of number of eggs to lifespan (in days) we can notice that flies from Chapecó had the best values at 20 and 30 °C (43.3 and

33.3 eggs per day against 29.7 and 5.71 for Curitiba, and 16.6 and 25.0 for Santa Maria). Flies from Curitiba and Chapecó performed better at 20 °C, while flies from Santa Maria performed better at 30 °C. This result may highlight the preference of Santa Maria for higher temperatures. Flies from Santa Maria had smaller eggs than the other populations (average of 0.05 mm<sup>3</sup>, comparing to 0.06 mm<sup>3</sup> from Chapecó and 0.07 mm<sup>3</sup> from Curitiba). Nevertheless, eggs from Curitiba and Chapecó had two times more variation (standard deviation = 0.01) than eggs from Santa Maria (standard deviation = 0.005), which suggests a more generalist strategies than Santa Maria. Moreover, reproductive traits showed less plasticity than non-reproductive traits.

In order to understand geographic patterns of thermal tolerance and adaptation is important to recognize that both mean temperature and temperature variation often vary along the distribution of species. Therefore, populations experience different annual mean temperatures and different degrees of temperature variation (Ragland & Kingsolver 2008). According to the climatic characterization, Curitiba and Chapecó are sites where the environment is considered more fine-grained and less seasonal. Thus, the individuals from these sites face an environment less predictable and with more variation – mainly in temperature – during their lifetimes. Furthermore, the mean annual temperatures of Curitiba and Chapecó are lower than Santa Maria.

A temperature 2 °C above the organism optimum temperature reduces the fitness more than a temperature 2 °C below the optimum (Martin & Huey 2008). If the mean temperature of environment equals the organism thermal optimum, both warming and cooling impair performance. While if mean temperature is below the organism thermal optimum, warming enhances performance more than cooling impairs performance (Bozinovic *et al.* 2011). However, thermal tolerance does not only depend on mean temperatures experienced by populations, but also on the variance of temperature. Overall, short-term environmental variance has the potential to impact life-history traits and fitness as much, or even more, than the mean temperatures alone do and can influence in the ecology and evolution of ectotherm life-history traits (Bozinovic *et al.* 2011; Folguera *et al.* 2011; Paaijmans *et al.* 2013). An increase in thermal variance could enhance fitness when the mean temperature of environment is below the thermal optimum (Martin & Huey 2008; Bozinovic *et al.* 2011).

The focal species of this study has its optimal temperature near 20 °C (Costa, Lecheta & Moura in prep.). Flies from Chapecó and Curitiba face mean annual temperatures almost 2 and 3 °C, respectively, below the optimal temperature and are localities with more short-term

temperature variation. Flies from Santa Maria are apparently acclimated in a region with mean temperatures near 20 °C – the optimum temperature for *S. chlorogaster* – and less short-term variation. Since flies from Santa Maria had the worst responses in life-history traits among temperatures, we can suggest that the mean temperatures and the environment variance could be enhancing the fitness for flies from Curitiba and Chapecó, even more in extreme temperatures. This is more notable when responses at 10 °C are analyzed, since flies from Santa Maria did not develop at this temperature, however is also evident in some traits at 20 and 30 °C.

Differences in thermal habitat among geographic populations of *S. chlorogaster* appear to have driven local adaptation. Since thermal tolerance is a result of mean temperature and variance experienced, when an environment (that could be represented by temperature) is more predominant, this may lead natural selection to canalize the best phenotypes, reducing plasticity (Braendle & Flatt 2006). As flies from Santa Maria have showed less plasticity in life history traits analyzed than the others populations and they are from a more climatic stable site, they showed a more thermal specialist strategy.

Additionally, flies from Curitiba and Chapecó are in the northern border of *S. chlorogaster* geographic distribution in Brazil. However, the difference in fitness among populations did not conform to a widespread assumption based on the theory of edge and central populations. This theory states that fitness should be reduced near the margin of the distribution, because these populations more frequently experience strong limiting factors (Sexton *et al.* 2009). Our results showed that flies from Curitiba and Chapecó (populations from the margin of the distribution) apparently had better fitness in almost all traits. The environmental characteristics in the border of *S. chlorogaster* distribution (colder region and higher temperature variance) probably selected a thermal generalist strategy in these populations lowering the effects of the limiting conditions encountered in this region. Nevertheless, to a better understanding of which factors are really operating in this response, further examination of adaptive genetic variation, dispersal limitation and dynamics of the populations must be performed together with more replicates from central population and the other margins of its distribution (Sexton *et al.* 2009).

The different climatic regimes among localities of the populations studied of *S. chlorogaster* is probably reflecting why, overall, flies from Santa Maria had the most different mean values along traits at different temperatures. Thus, we can suggest that natural selection is modulating life-history traits of these populations, leading to adaptive phenotypic plasticity.

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### **CAPÍTULO III**

Thermal tolerance in *Sarconesia chlorogaster* (Diptera, Calliphoridae)

## INTRODUCTION

In ectotherms, temperature has a major effect on physiological processes, which in turn influences the fitness. For example, temperature can affect metabolic rate, locomotion and life-history traits and changes in these aspects directly affect the organism performance (Angilletta 2009; Castañeda *et al.* 2005). Temperature does not only affect in an individual scale, but also drives patterns of local adaptation (Chown 2001; Hoffmann 2010). Physiological adaptation allows ectotherm organisms to tolerate a range of temperatures, which is an important factor that can determine their geographic distribution (Hoffmann *et al.* 2003; Fallis *et al.* 2014; Andersen *et al.* 2015).

Generally, in the study of the thermal biology of ectotherms, two aspects are highlighted: temperature tolerance and temperature-dependent effects on performance. Although that thermal limits are influenced by several aspects (e. g., life stages, age - Bowler & Terblanche 2008) the thermal tolerance of an individual is related to both organisms and the thermal environment in nature to which they are typically exposed (Schulte *et al.* 2011; Hoffmann *et al.* 2013). The effects of temperature on performance can be visualized using a thermal performance curve (Fig. 1), in which the y-axis is the performance and the x-axis is the variable (temperature) (Hoffmann 2010; Schulte *et al.* 2011). Generally, the thermal optimum of an organism (where its performance is maximized) lies many degrees above the lower limit of thermal tolerance but only a few degrees below the upper limit (Bozinovic *et al.* 2011). Therefore, the thermal performance curves tend to have the same general shape, with the performance typically increasing as temperature increases, and decreasing rapidly after the maximum is reached at some intermediate temperature (Angilletta 2009; Schulte *et al.* 2011). Besides determining the optimal temperature for the organisms, the knowledge of thermal tolerance of populations and species is also important, since it may provide insights about the local adaptation, factors that could be limiting the geographic distribution and in the context of global warming.

Organisms respond differently when subject to short versus long exposure to extreme temperatures, and the responses can vary from reversible physiological changes to death. Furthermore, the effect of the exposition to extreme temperatures vary if the individual was previously exposed to non-lethal but extreme temperatures for a certain period of time (Hoffmann *et al.* 2003).

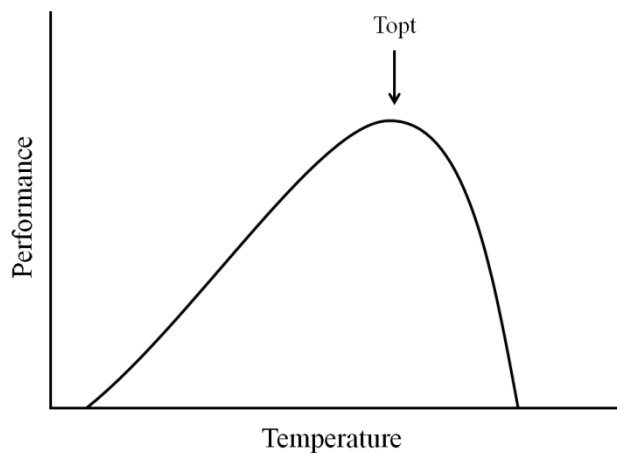
Thermal tolerance can be measured for lower and upper temperatures and the study of thermal tolerance can be done in a variety of ways. One of them is to examine the intrinsic

tolerance, the exposure of organisms to extreme temperatures in the absence of a preparatory temperature treatment (Yoder *et al.* 2006). Usually, extreme temperatures can be tolerated only for a short period. In this context, we can expose the organism to a cold or heat shock for a short period and examine how it responds after it (for instance, survivorship, recovery time, reproduction). Generally, the goal of these experiments is to analyze the performance, thus, the dependent variable (the response of the individuals) is linked with fitness. Among different procedures, the response after a cold shock can be measured by chill coma induction followed by the time that the organism takes to recover. This kind of assay is a widely used metric for adults of *Drosophila* (Hoffmann *et al.* 2003; Sinclair & Roberts 2005) because it is non-lethal and the responses variation may correspond to geographic variations (Gibert *et al.* 2001). Similarly to cold shocks, different experimental protocols can be used to determine the response after a heat shock. One way is to determine the survival some hours after a heat shock (Goto & Kimura 1998; Nielsen *et al.* 2005; Hu *et al.* 2014). The heat shock response also may reflect the evolutionary background of an organism. Therefore, we expect the specific patterns of the heat shock response to depend on the actual and past thermal conditions of the organism (Bährndorff *et al.* 2009).

For insects, there is evidence for an association between lower thermal limits and latitude (Gibert *et al.* 2001; Hoffmann *et al.* 2002). Overall, populations from high latitudes face lower temperatures than populations from low latitudes and, usually, populations from localities with lower mean temperatures recover faster from chill coma (Gibert *et al.* 2001; Hallas *et al.* 2002; Hoffmann *et al.* 2002; Castañeda *et al.* 2005; Fallis *et al.* 2014). For upper thermal limits, the results are mixed, with evidence supporting both, a strong association between upper limit and latitude and a weak association between them, mainly depending on experimental design (Addo-Bediako *et al.* 2000; Hoffmann *et al.* 2002). Additionally, there is evidence that the upper thermal limits show considerably less geographic variation than the lower thermal limits (Addo-Bediako *et al.* 2000). Besides latitude, thermal limits also may be dependent on altitude and the environment variation regime that the organism faces during its lifetime (Pétavy *et al.* 2001; Folguera *et al.* 2008). In this context, altitude and environment can be associated, since thermal amplitude is positively correlated with altitude (Folguera *et al.* 2008). There is evidence that more variable environments increase the thermal tolerance breadth, since plasticity may be favoured when environmental fluctuation is frequent (Folguera *et al.* 2008; Bozinovic *et al.* 2011; Fallis *et al.* 2014).

*Sarconesia chlorogaster* (Calliphoridae) has its geographic distribution in South America, mainly associated with colder regions (James 1970; Dear 1979; Carvalho & Ribeiro 2000). Previous studies showed that *S. chlorogaster* has a good tolerance for cold temperatures but is sensitive to warmer temperatures (Lecheta *et al.* 2015). This species also shows different responses to temperature among populations across a small range of latitude (cap. II). Among the populations we used in this study, one is characterized by having less short-term temperature variation (more predictable environment) and higher temperature means throughout the year, comparing with the other two populations (cap. II).

In this work, we test if thermal tolerance differs among populations of *S. chlorogaster* in a small latitudinal gradient. We measure cold tolerance using a chill coma recovery time assay and heat tolerance after 24 hours of exposed to extreme heat. We hypothesize that differences among environments will select for different upper and lower limits among populations. We expect that populations from more variable and colder environments will show higher tolerance to extreme temperatures than the population from warmer and predictable environment.



**Figure 1.** Hypothetical thermal performance curve. The temperature at which performance is maximized is termed as  $T_{opt}$ . The points at which performance is zero are the lower and upper limits of thermal tolerance.

## MATERIAL AND METHODS

### Collection and maintenance of *S. chlorogaster*

We sampled three populations of *S. chlorogaster* in south of Brazil, Curitiba (25° 26' S), Chapecó (27° 03' S) and Santa Maria (29° 41' S), in 2013 and 2014 and used these samples to start the colonies (Fig. 2). Populations were named after the city where sampling

took place. Flies were collected using a trap with rotting sardines as bait. In each location, a minimum of 20 flies (males and females) were collected.

The flies were maintained from egg to adult under common-garden conditions (at 25 °C and on a light/dark cycle of 12h) until the heat/cold shock treatment (up to 20 generations). Adults were fed with sugar, milk powder, raw ground beef and water *ad libitum* and larvae were fed with diet modified from Estrada *et al.* (2009). All experiments used 5- to 7-day old flies with unknown reproductive history, separated by sex in order to account also for sex-specific differences in phenotype.

### **Recovery time from cold shock**

To determine recovery times, adults from the stock colony were transferred to empty 1.5 mL polypropylene tubes (one fly per vial) and immediately allocated into a freezer at -5 °C for 30 minutes. Freezer temperature was monitored with a thermohygrometer during all the experiment. After 30 minutes, flies were in chill coma and unable to move. These adults were placed at room temperature (approx. 20 °C) to measure recovery time for up to 20 min. Flies that did not recover within 20 minutes were excluded from the analysis. The flies were considered recovered from chill coma when they could stand on their legs. We measured recovery time as the time (in minutes) elapsed from the moment they were out of the freezer until they could stand. Thirty males and thirty females were cold shocked per population, spread across three days (10 males and 10 females per population per day). No flies were used more than once.

### **Heat shock tolerance**

Survivorship after a heat shock was assessed with adults from the stock colony. These adults were transferred to empty 1.5 mL polypropylene tubes (one fly per vial) and immediately submerged in a water bath at 40 °C for 90 minutes. Temperature was monitored with a thermometer during all the experiment. After 90 min, all flies were placed into a small cage at room temperature (approx. 20 °C) and allowed to recover for 24 hours. Survivorship was measured by counting the flies that were alive in the cage by the end of these 24 hours. Thirty males and thirty females were heat shocked per population (10 males and 10 females per day over three days). We never measured the same fly twice.

### Climatic characterization of populations

A complete climatic characterization of the populations' sites used in this study is given in cap. II. Briefly, the climatic characterization is clearly different between the city of Santa Maria and the cities of Curitiba and Chapecó. Santa Maria has lower isothermality, higher annual mean temperature and lower altitude when compared to Curitiba and Chapecó.

### Statistical analysis

All the analysis was made on R environment v3.2.2 (R Core Team 2015). In order to determine population and sex effects on the recovery time after the cold shock, we performed a two-way analysis of variance (ANOVA). To test population and sex effects on fly survival 24 hours after the heat shock, we fitted a generalized linear model (GLM) assuming binomial errors. When our analyses yielded significant results, we performed specific post hoc comparisons between pairs of populations and sex. Prior to analyses we checked assumptions of normality and homoscedasticity of raw data. A level of  $p < 0.05$  was used to reject the null hypothesis. For the GLM subsequent analysis lsmeans library (Russell 2015) was used.



**Figure 2.** Populations sites (Brazil) used to start the colonies of *Sarconesia chlorogaster*.

## RESULTS

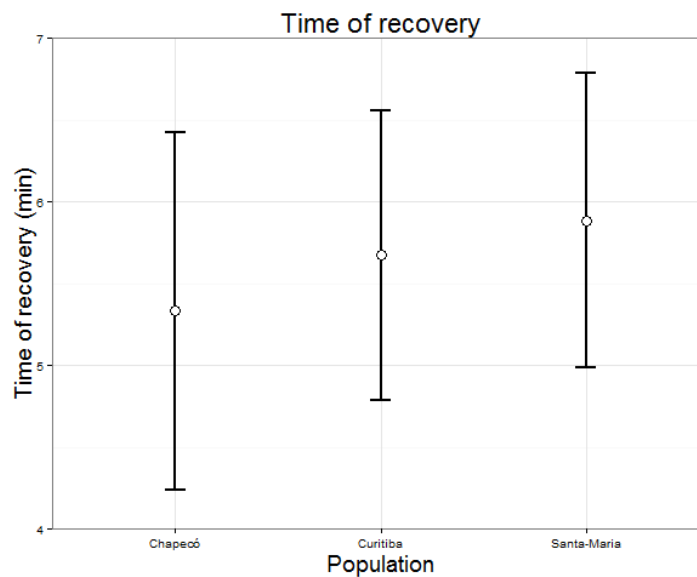
### Cold shock

Time of recovery from chill coma was significantly different among populations ( $F_{2,156} = 4.38$ ,  $p < 0.05$  – Fig. 3). Flies from Chapecó recovered faster ( $5.33 \pm 1.09$  min),

followed by flies from Curitiba (5.68  $\pm$  0.88 min) and Santa Maria (5.89  $\pm$  0.90 min). However, recovery time from flies from Curitiba was not significantly different from flies from Chapecó and Santa Maria. Time of recovery of flies was not affected by sex ( $F_{1,156} = 0.96$ ,  $p = 0.33$ ) and there was no significant interaction between sex and population ( $F_{2,156} = 0.06$ ,  $p = 0.94$ ).

### Heat shock

As well as in the cold shock experiment, survival after the heat shock was significantly different among populations ( $X^2_2 = 10.1$ ,  $p < 0.01$ ). Flies from Curitiba had the best percentage of survival (78.3%), followed by flies from Santa Maria (56.6%) and Chapecó (53.3%). Survival was not significantly different between flies from Chapecó and Santa Maria. The sex of individuals did not affect survival ( $X^2_1 = 3.05$ ,  $p = 0.08$ ) and, also, there was no significant interaction between sex and population ( $X^2_2 = 1.66$ ,  $p = 0.44$ ).



**Figure 3.** Time of recovery from chill coma. The y-axis is the time of recovery in minutes, while the x-axis is the population of origin. Mean  $\pm$  standard deviation.

### DISCUSSION

Thermal tolerance studies among populations can give insights about the mechanisms underlying the geographic distribution of a species and about the local adaptation of its populations. Differences among populations are more evident when comparing across a large range of latitude. However, even near populations can show differences in thermal responses

and local adaptation. Here, we examined the chill coma recovery time and survival after a heat shock among three *S. chlorogaster* populations collected along a small latitudinal gradient in Brazil. Our major finding was that recovery time and survival varies among populations. Nevertheless, the response between heat and cold shocks did not have the same direction across populations.

Generally, there is a latitudinal pattern of cold tolerance, the higher the latitude the higher the tolerance to cold (Hallas *et al.* 2002; Castañeda *et al.* 2005). This is because, overall, populations from high latitudes have lower temperatures throughout the year. However, at the latitudinal scale that we used, this pattern cannot be considered, since the population from higher latitude has higher temperatures than the other two localities, probably because of the altitudinal differences.

Populations from localities with lower mean temperatures usually recover faster from chill coma than populations with higher mean temperatures and this could reflect a possible directional selection on the lower thermal tolerance caused by the constant exposition to cold (Gibert *et al.* 2001; Hallas *et al.* 2002; Hoffmann *et al.* 2002; Castañeda *et al.* 2005; Fallis *et al.* 2014). We expected this pattern to hold for *S. chlorogaster*. Contrary to that expectation, recovery time from chill coma was not faster for flies from Curitiba, the population with the lowest annual mean temperature among the populations studied (17.1 °C against 18.6 °C and 19.4 °C for Chapecó and Santa Maria, respectively – Hijmans *et al.* 2005). However, flies from Santa Maria recovered slower than the other populations, a result expected from this model. Curitiba showed a mixed response not corresponding to any previous expectations.

Aside from latitude, variation in plasticity, or the ability to be more or less tolerant to changes in temperature, is also associated with the altitude of the population of origin and how much the environmental is variable (Folguera *et al.* 2008; Bozinovic *et al.* 2011; Fallis *et al.* 2014; cap. II). Usually, the higher the altitude the lower the mean temperature. Therefore, we could expect from individuals from higher altitudes to be more tolerant to cold. Furthermore, populations living at different altitudes are not only exposed to different mean temperatures but also experience different short-thermal regimes, and thermal amplitude is often positively correlated with altitude (Folguera *et al.* 2008). Thus, the combination of altitude and short-term variation may be important to determine the thermal tolerance of each population (Folguera *et al.* 2008; cap. II). Curitiba and Chapecó have altitudes of more than 650 m (890 m and 670 m, respectively) while Santa Maria has a lower altitude (110 m) (Hijmans *et al.* 2005). Additionally, Santa Maria has less short-term temperature variation (a



more predictable environment) than Curitiba and Chapecó. Our results showed that flies from Chapecó recovered faster than flies from Santa Maria. This is consistent with the assumptions that populations from high altitudes and variable thermal environmental have their fitness increased (Folguera *et al.* 2008; Fallis *et al.* 2014). However, although Curitiba has the highest altitude and a short-term thermal variation similar to Chapecó, the recovery of flies from Curitiba did not showed the expected trend. Hence, maybe our analysis does not have enough statistical power to detect significant differences among populations or there is a significant interaction underlying the variation in chill coma recovery time that we were not able to detect with this experiment. Moreover, we did not find significant difference in recovery time between sex and neither a significant sex and population interaction.

Heat tolerance can increase when organisms develop under warmer conditions (Hoffmann *et al.* 2003; Cooper *et al.* 2008). Thus, we could expect from populations from a locality that has higher annual mean temperature (Santa Maria population) to have higher resistance to heat shock. However, flies from Curitiba had the best percentage of survival after the heat shock, almost 80% of adults survived against approximately 55% from Santa Maria and Chapecó. This result agrees with other studies that showed that previous conditions before an exposure to heat stress may not enhance upper thermal tolerance (Goto & Kimura 1998; Boher *et al.* 2012; Hoffmann *et al.* 2013). Nevertheless, our results showed that flies from the coldest locality had the best survival rate after a heat shock. Since exposure to colder temperatures can also help in a better response to warmer temperatures (cross resistance) (Hoffmann *et al.* 2003; Sinclair & Roberts 2005), it is possible that the exposition of flies from Curitiba to colder temperatures enhanced their thermal tolerance to warmer temperatures. We can also suggest that this population may have a different heat shock proteins expression, which may enhance their thermal tolerance. Furthermore, resistance to heat shock may have less plasticity than resistance to low temperatures (Chown 2001; Alford *et al.* 2012). Plastic changes increase thermal limits in many terrestrial ectotherms, but tend to have less effect on upper limits than lower limits, thus, upper limits tend to vary less (Hoffmann *et al.* 2013). The response of flies from Santa Maria and Chapecó seems to agree with this hypothesis, since they showed different recovery times from chill coma but the same survival rate after a heat shock. As well as we found in the chill coma recovery time experiment, we did not find significant difference in survival after the heat shock between sex and neither a significant sex and population interaction.

Recovery time from a chill coma is an estimator of cold tolerance in terrestrial arthropods and appears to be very informative and an easy index to describe thermal adaptation (Hoffman *et al.* 2002; David *et al.* 2003; Castañeda *et al.* 2005). Likewise, survival after a heat shock is one way to understand how ectotherms deal when exposed to an extreme elevated temperature. In this study, we showed differences in thermal responses among three populations of *S. chlorogaster* using these two assays. Our results suggest that upper thermal tolerance is more conserved than lower thermal tolerance. Additionally, we found a relationship between short-term environment and lower limit for two populations. The population from Curitiba did not fit any prediction, showing similar responses to both, heat and cold shock. We suggest that further studies must be done with more populations and addressing different approaches, for instance analyzing the expression of heat shock proteins, in order to understand the mechanisms behind thermal tolerance variations among populations of *S. chlorogaster*. Moreover, this is the first work that explores geographic variation after a cold and heat shock in a Calliphoridae species.

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