Universidade de São Paulo
Instituto de Biociências
Departamento de Fisiologia
Programa de Pós-Graduação em Ciências Biológicas- Fisiologia Geral

Sensibilidade e tolerância térmica da mosca-das-frutas
Anastrepha sp.1 aff. fraterculus (Diptera: Tephritidae)

Thermal sensitivity and thermal tolerance of the fruit-fly
Anastrepha sp.1 aff fraterculus (Diptera: Tephritidae)

Stefane Saruhashi

São Paulo

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Dissertação apresentada ao Instituto de Biociências da Universidade de São Paulo, para a obtenção de Título de Mestre em Ciências, na Área de Fisiologia Geral.

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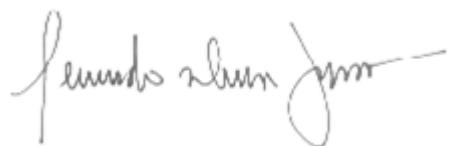
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Dedicatória

Dedico este trabalho à minha família que, com muito carinho e paciência me apoiaram nessa jornada.

Bete, Nelson e Tati

Epígrafe

"Turn your face to the Sun and the shadows will fall behind you"

- Provérbio Maori -

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Índice

Resumo Geral

Temperaturas extremas podem causar danos celulares sendo potencialmente letais para os organismos. Faixas de temperatura corpórea (Tb) intermediárias normalmente garantem o desempenho máximo do organismo e os extremos de Tbs podem causar danos letais para os organismos. As relações entre a temperatura corpórea e processos termo-dependentes de organismos ectotermos podem ser descritas por curvas de desempenho. Dada sua relevância na determinação do valor adaptativo de animais, o desempenho locomotor tem sido frequentemente usado para determinar o efeito da Tb sobre diferentes níveis de organização. Como insetos dependem majoritariamente de seu metabolismo aeróbico para suprir a demanda energética, a atividade de enzimas que compõem a via do metabolismo aeróbico pode ser determinante para o desempenho locomotor. Assim, essa dissertação teve como objetivo investigar as inter-relações entre limites térmicos e sensibilidade térmica do desempenho locomotor, locomoção voluntária e capacidade aeróbica mitocondrial de uma espécie de mosca-das-frutas a *Anastrepha sp.1 affinis fraterculus*. Nossos resultados mostraram que o desempenho locomotor forçado pode ser descrito por uma curva com formato de U e deslocada para a esquerda, com temperatura ótima de ~ 28.7^{o}C, temperatura crítica mínima de ~3.9^{o}C e máxima de ~ 43.1^{o}C. As moscas apresentaram menor frequência de locomoção voluntária se comparada com a locomoção forçada, mas andaram mais dentro da faixa de 18 a 23°C. Quanto à capacidade aeróbia, a atividade enzimática da CS aumentou com o aumento da temperatura até a margem crítica dos 43°C – e portanto não deve ser considerada como uma questão restritiva para o desempenho locomotor.. Por fim, não identificamos uma redução do desempenho máximo contrapondo a ampliação da faixa ótima de funcionamento locomotor, o que refuta a hipótese '*jack-of-all temperatures is a master of none*'.

Palavras chave: desempenho locomotor, limites térmicos, capacidade aeróbica, mosca-das-frutas, curva de desempenho térmico.

General Abstract

Extreme temperatures can cause damage to cell phones, being potentially lethal to organisms. Normally guaranteed intermediate temperature (Tb) boxes or maximum organism performance and Tbs extremes can cause lethal damage to organisms. The relationships between body temperature and the thermo-dependent processes of ectothermic organisms can be shown by variations in performance. Its relevance in determining the adaptive value of animals, locomotor performance has often been used to determine the effect of Tb at different levels of organization. As insects rely mostly on their aerobic metabolism to supply an energy demand, an activity of enzymes that make up the aerobic metabolism pathway can be determinant for locomotor performance. Thus, this dissertation aimed to investigate how interrelationships between thermal limits and thermal sensitivity of locomotor performance, voluntary locomotion and mitochondrial aerobic capacity of a fruit fly species in Anastrepha sp.1 affinis fraterculus. Our results show that the performance of the locomotive engine can be described by a U-shaped curve shifted to the left, with an ideal temperature of ~ 28.7oC, a minimum critical temperature of ~ 3.9oC and a maximum of ~ 43.1oC. As the flies showed a lower frequency of voluntary locomotion compared to forced locomotion, more walked more within the range of 18 to 23oC. As for aerobic capacity, an enzymatic activity of CS increased or increased in temperature up to a critical margin of 43oC - therefore, it should not be considered as a restrictive issue for locomotor performance. Finally, it does not identify a reduction in maximum performance, in contrast to an expansion of the optimum range of locomotor operation, refuting the 'Jack-of-all-temperatures is a master of none' hypothesis.

Keywords: Locomotor performance; thermal limits; aerobic capacity; fruit fly; thermal performance curve.

Introdução Geral

1. O efeito da temperatura sobre o organismo

O cenário de mudanças climáticas atual prevê um aumento na temperatura média da superfície continental e oceânica (Sánchez-Lugo, *et al.*, 2018; IPCC, 2018), bem como um aumento na frequência e intensidade de eventos climáticos extremos e imprevisíveis (Thornton, 2014; IPCC, 2012). Essas alterações térmicas podem promover mudanças em diferentes níveis de organização dos animais, influenciando desde funções proteicas (Somero, 1995; Hochachka & Somero, 2002; Angilletta, 2009) até interações ecológicas, como observado na interação entre herbívoros e plantas (O'Connor, 2009). Além disso, a exposição às temperaturas extremamente baixas ou altas podem gerar danos celulares (Sørensen *et al.,* 2013; Toxopeus & Sinclair, 2018) e são potencialmente letais (Lencioni & Bernabò, 2017). Em pequenos invertebrados ectotérmicos como insetos, as alterações térmicas do ambiente são especialmente relevantes devido a sua baixa inércia térmica, tornando-os sujeitos a rápidas alterações de sua temperatura corpórea (McCue & De Los Santos, 2013; Kemp & Krockenberger, 2004). Entretanto, insetos contam com estratégicas comportamentais (e.g. *sun basking* e agregação de formigas *Formica polyctena,* Kadochová *et al.,* 2019; *bubbling* da mosca *Crysomya megacephala,* Gomes *et al.*, 2018) e mecanismos autonômicos (e.g. bombeamento de hemolinfa do tórax para o abdômen durante o vôo da mariposa *Manduca sexta*, Heinrich, 1970) que permitem a manutenção de sua temperatura corpórea dentro de uma faixa térmica tolerável.

A relação entre a temperatura corpórea e processos termo-dependentes, como a taxa metabólica (Schulte, 2015), contração muscular (Bennet, 1985) e taxa de desenvolvimento (Shi & Ge, 2010), podem ser descritos por uma curva de desempenho térmico (*Thermal performance curve – TPC* (Huey & Stevenson, 1979) (Fig.1). A *TPC* é caracterizada por uma fase ascendente, cuja inclinação é determinada por efeitos termodinâmicos (Rezende & Bozinovic, 2019; Schulte, 2015). Nesta fase, o desempenho aumenta conforme a temperatura. Entretanto, em temperaturas superiores à temperatura ótima (T_o), processos de desnaturação proteica ganham importância, havendo uma rápida diminuição do desempenho (Rezende & Bozinovic, 2019; Schulte, 2015; Angilletta, 2009). Ao determinar a forma da *TPC*, é

possível identificar alguns parâmetros importantes como: o T_o, temperatura em que o desempenho é maximizado; os limites térmicos do desempenho ($T_{br} = CT_{max} - CT_{min}$) e medidas arbitrarias como a faixa térmica em que 80% do desempenho máximo é atingido (B_{80}). Com esses parâmetros, é possível comparar as tolerâncias térmicas em diferentes populações (Phillips *et al.*, 2014), processos que ocorrem em diferentes níveis de organização (*e.g.* taxas de fotossíntese, velocidade de corrida e crescimento populacional, Rezende & Bozinovic, 2019; Phillips *et al.*, 2014) e testar hipóteses que permeiam o campo da fisiologia termal.

Em nível de organismo, a *TPC* está restrita a uma faixa térmica operacional delimitada pelas temperaturas críticas mínima e máxima do organismo. Estas temperaturas críticas são diagnosticadas por uma série de comportamentos prévios à morte do animal. A exemplo disto, quando a temperatura corpórea de um inseto diminui, ocorre a redução progressiva de sua locomoção, seguida pela perda de sua coordenação motora e o animal entra em um estado coma reversível. Esta temperatura em que esse fenômeno ocorre é considerada como temperatura crítica mínima (CT_{min}) (MacMillan & Sinclair, 2011; Hazell & Bale, 2011, Schou *et al.*, 2017). Apesar do animal em coma não conseguir realizar atividades relevantes, ele pode recuperar suas funções quando exposto a temperaturas mais altas (Sinclair *et al.*, 2015). O aumento da temperatura, por sua vez, aumenta a atividade locomotora do animal, mas em temperaturas próximas do limite térmico ocorre a perda de respostas posturais e ocorrência de espasmos musculares. Por fim, o animal entra em um estado de coma, caracterizado como sua temperatura crítica máxima (CT_{max}) (Lutterschidt & Hutchison, 1997). Neste caso, a exposição ao CT_{max} demanda um período maior de recuperação, como observado em formigas *Atta sexdens rubropilosa*, as quais apresentaram uma menor tolerância ao calor por 6 dias depois da exposição à sua CT_{max} (Ribeiro *et al.*, 2012).
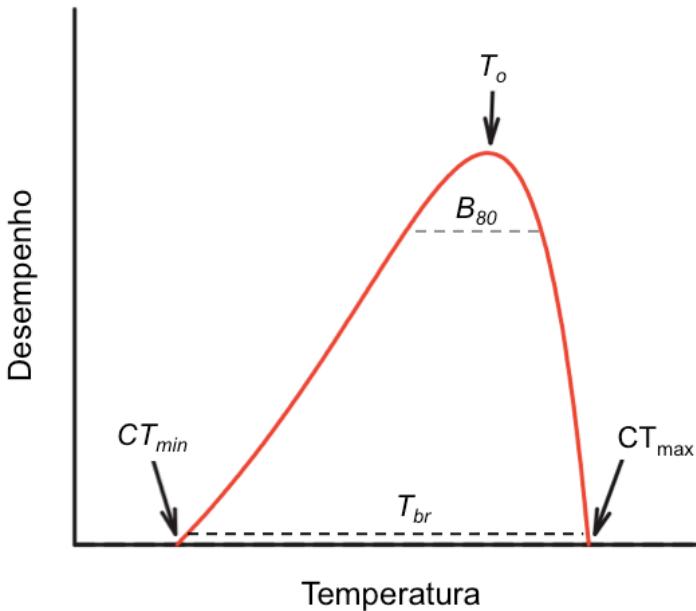
Figura 1. Curva de desempenho térmico de animais ectotermos. A linha vermelha descreve a relação entre o desempenho e temperatura corpórea. As setas indicam os parâmetros da curva: temperatura ótima (T_o), na qual o desempenho é máximo, e temperatura críticas mínima (CT_{min}) e máxima (CT_{max}), nas quais o desempenho é zero. A linha tracejada preta indica a amplitude térmica de desempenho (T_{br}) e é delimitada pelas temperaturas críticas. A linha tracejada cinza representa a amplitude térmica em que o animal atinge 80% de seu desempenho máximo (B_{80}). Figura adaptada de Krenek *et al.*(2011).

Dentre as hipóteses que permeiam o campo da fisiologia termal, a hipótese "*jack-of-all-temperatures is a master of none*" (Fig. 2a) destaca-se como uma das mais influentes e controversas. De acordo com essa hipótese, existe um compromisso entre o desempenho máximo de uma função e a faixa térmica em que esta pode ser desempenhada (Huey & Hertz,1984). Assim, indivíduos (ou espécies) que possuem um fenótipo especialista, podem atingir um desempenho elevado dentro de uma faixa térmica restrita, mas não são capazes de manter o mesmo desempenho fora dela. Enquanto isso, animais com fenótipos generalistas são capazes de operar em uma grande faixa térmica, mas com desempenho comparativamente menor (Angilletta, 2009). Desta forma, a *TPC* de um especialista é mais estreita, enquanto a *TPC* de um generalista é mais larga e tem menor amplitude. Apesar dessa hipótese ser prevista pela teoria (Gilchrist, 1995), estudos empíricos nem sempre corroboram essa hipótese, como observado em lagartos (Huey & Hertz,1984; Bennett, 1980), bactérias do gênero *Amoeba* (Huey & Hertz,1984), crustáceos da espécie *Daphnia pulicaria* (Palaima & Spitze, 2004) e peixes teleósteos (Nati *et al.,*2016). Segundo Huey e Hertz (1984), indivíduos que desempenham suas atividades em uma ampla faixa térmica e

não tem prejuízo no seu desempenho seguem o padrão descrito pela hipótese "*jack–off-all-temperatures is a master of all*" (Fig. 2b). Neste caso, os indivíduos com maior desempenho máximo teriam uma *TPC* com maior amplitude e mais larga, enquanto os indivíduos com menor desempenho máximo apresentariam uma *TPC* com menor amplitude e mais estreita.
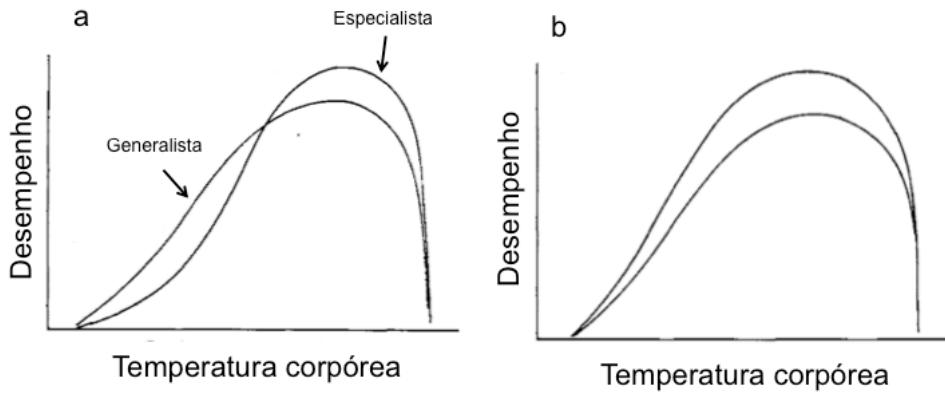
Figura 2. Curvas de desempenho em função da temperatura corpórea. (a) Descreve a hipótese "*Jack-of-all-temperatures is a master of none*" em que há um compromisso entre o desempenho e amplitude térmica do desempenho. As setas indicam a curva que descreve um animal com fenótipo generalista e um especialista. (b) Descreve a hipótese "*Jack-of-all- temperatures is a master of all*" em que não existe um compromisso entre desempenho e amplitude térmica de desempenho. Figura adaptada de Huey e Hertz (1984).

A fase ascendente e a fase descendente da *TPC* estão relacionadas ao efeito da temperatura sobre as taxas de reação e desnaturação de proteínas, respectivamente (Rezende & Bozinovic, 2019; Schulte, 2015). Enzimas possuem um papel fundamental sobre o metabolismo energético, dado que elas diminuem a energia necessária para ativação de reações químicas permitindo que ocorram em altas taxas em temperaturas biologicamente relevantes (Hochachka & Somero, 2002). Como o metabolismo energético é composto por um conjunto de reações bioquímicas celulares mediadas por enzimas (Randall *et al.,* 2000), é coerente esperar que o metabolismo energético com um todo também é influenciado pela temperatura. Como observado em lagartas sub-antarticas *Pringleophaga marioni* (Sinclair *et al.,* 2004)*,* em grilos *Hophlosphyrum griséus* (Nespolo, 2013) e percevejos *Cimex lectularius* (DeVries *et al.*, 2013).

Além do metabolismo energético, mudanças em taxas de outras reações bioquímicas por elevação da temperatura também repercutem em alterações comportamentais. Em joaninhas da espécie *Coleomegilla maculata*, por exemplo, o

aumento da temperatura corpórea acelera sua taxa de digestão e reduz o tempo de manuseio das presas (Sentis *et al.*, 2013). Além disso, a termocepção e integração da informação no sistema nervoso central também induzem mudanças comportamentais dependentes da temperatura corpórea (Abram *et al.*, 2016). Formigas da espécie *Myrmecia croslandi* mudam seu padrão de forrageio contínuo para um padrão bimodal quando a temperatura ambiental ao meio dia supera 35°C (Jayatilaka *et al.*, 2011). Adicionalmente, as temperaturas em que insetos holometábolos são expostos durante seu desenvolvimento afetam o desempenho do animal na fase adulta. Cavieres e colaboradores (2016), por exemplo, verificaram que moscas adultas de *Drosophila melanogaster* que se desenvolveram em temperaturas altas (28°C e 28°C±4°C) atingiram velocidades de caminhada maiores em altas temperaturas (32 a 44°C) em comparação a indivíduos que se desenvolveram em temperaturas baixas (15°C e 15±4°C).

Além das hipóteses "*jack-of-all-temperatures is a master of none*" e "*jack-of-all-temperatures is a master of all*" outras hipóteses que permeiam o campo da fisiologia térmica avaliam o efeito da aclimatação como fator determinante para sensibilidade térmica do animal. A hipótese da aclimatação benéfica pressupõe que a aclimatação leva à mudanças adaptativas do organismo. Assim, animais aclimatados tem vantagem sobre outros quando são expostos à faixa de temperatura em que foram aclimatados (Leroi *et al.*, 1994). Contudo, tanto estudos empíricos (Gilchrist & Huey, 2001), quanto o próprio estudo de Leroi e colaboradores (1994) refutam a abrangência dessa hipótese ao identificarem organismos aclimatados a outras temperaturas com um desempenho superior ao de organismos aclimatados às condições testadas. Huey e colaboradores (1999) enfatizaram a importância de testar múltiplas hipóteses ao abordar respostas de aclimatação. Dentre essas hipóteses temos a "*Colder is better*" inicialmente introduzida como "*Bigger is better*", a qual pressupõe a existência de uma vantagem ecológica associada ao tamanho corpóreo do animal, e que ambientes frios favorecem o desenvolvimento de animais maiores (Huey & Berrigan, 1996). Além disso, a hipótese "*Hotter is better*" parte da premissa de que altas temperaturas corpóreas inevitavelmente aumentam as taxas de reações bioquímicas e assim o organismo apresenta um desempenho maior em sua T_o (Knies *et al.*, 2009; Angilletta *et al.*, 2009), indo contra a hipótese "*Colder is better*". Já a hipótese ótima de desenvolvimento assume que animais aclimatados à temperaturas intermediárias apresentam um desempenho maior em qualquer ambiente (Huey & Berrigan, 1996).

Por fim, temos a hipótese do amortecimento do desenvolvimento, a qual considera que a temperatura de desenvolvimento do animal não afeta seu desempenho quando adulto (Huey & Berrigakn, 1996). Sendo assim, diversas hipóteses tem sido formuladas e são passíveis de serem testadas através de estudos comparativos e experimentais envolvendo parâmetros derivados da curva de sensibilidade térmica.

2. Paradigma do desempenho e locomoção

Segundo o "paradigma de desempenho" proposto por Arnold (1983), a seleção natural atua sobre o desempenho do organismo e desta forma influencia seu valor adaptativo. Dado que o desempenho é determinado por caracteres de níveis hierárquicos inferiores (*e.g.* traços morfológicos, processos fisiológicos e bioquímicos), a seleção natural promove uma evolução integrada de múltiplos níveis de organização ao atuar sobre o desempenho (Arnold, 1983). Assim, podemos dividir o efeito da seleção natural em dois gradientes: o gradiente de desempenho e o de aptidão. Estudos associados ao gradiente de desempenho seriam aqueles cujo objetivo é verificar a influência de diferentes caracteres sub-organismais sobre o desempenho, enquanto através da análise do gradiente de aptidão seria possível verificar a influência do desempenho sobre o valor adaptativo do organismo (Arnold, 1983). Garland e Carter (1994) acrescentaram a esse paradigma a perspectiva de que a seleção natural atua majoritariamente sobre o comportamento do animal, que por sua vez é limitado pelo seu desempenho.

O desempenho locomotor dos animais tem relação com diversos determinantes da aptidão, como, por exemplo, nas estratégias anti-predatórias em aranhas *Hogna carolinensis* (Nelson & Formanowicz, 2005) e na taxa de exibição comportamental (*e.g.* flexões, extensão da papada, deslocamento lateral, perseguição e mordida) em lagartos machos territoriais de *Anolis cristatellus*, cujo resultado da disputa influencia seu sucesso reprodutivo (Perry *et al.*, 2004). Além disso, características morfológicas estão intimamente relacionadas ao desempenho locomotor dos organismos, como observado em sapos *Rhinella marina*, cujos indivíduos com pernas mais longas foram mais rápidos e os primeiros a invadirem novas áreas na Austrália (Phillips *et al.*, 2006). Ademais, estudos mostram a relação entre o desempenho locomotor de artrópodes e a temperatura ambiental. Moscas da espécie *Drosophila melanogaster*, por exemplo, caminharam com maior velocidade

em temperaturas superiores a 18°C e em baixas pressões atmosféricas, bem como mostraram-se mais motivadas a voarem em temperaturas entre 25 e 30°C (Dillon & Frazier, 2006). Assim, ao estudarmos o efeito da temperatura sobre o desempenho locomotor do animal, pode ser possível compreender a influência da temperatura sobre diferentes níveis de organização.

A atividade de enzimas que compõem a via do metabolismo aeróbico pode ser determinante para o desempenho locomotor dos insetos. A citrato sintase (CS) destaca-se por ser altamente conservada e por limitar o primeiro passo do ciclo de Krebs ao catalisar moléculas de acetilcoenzima A (acetil-CoA) e ácido oxalacético, formando citrato (Remington, 1992). Dado que insetos dependem majoritariamente de seu metabolismo aeróbico para suprir a demanda energética de suas atividades locomotoras (Full & Tullis, 1990; Suarez, 2000). Além disso, a atividade de CS parece aumentar com o ganho de experiência de forrageio de abelhas *Apis melífera* (Schippers *et al.,* 2006). Desta forma, a sensibilidade térmica dessa enzima pode ser fundamental para suprir a demanda energética e sustentar a atividade locomotora de insetos em diferentes temperaturas.

Em geral, outras características como tamanho e massa corpórea também podem determinar o desempenho locomotor de artrópodes. Além disso, no caso dos artrópodes, o dimorfismo sexual está muitas vezes atrelado ao tamanho dos animais, como observado em escorpiões machos de *Centruroides vittatus,* os quais têm apêndices locomotores maiores e são mais velozes do que as fêmeas co-específicas (Carlson *et al.*, 2014). Entretanto, o dimorfismo sexual e desempenho locomotor é variável. Vespas fêmeas *Aphidius ervi* são maiores e mais velozes que machos co-específicos (Gilchrist, 1996), enquanto fêmeas das espécies de pulgas do deserto *Xenopsylla conformis mycerini, Xenopsylla ramesis, Xenopsylla dipodilli, Parapulex chephrenis, Synosternus cleopatrae pyramidis* e *Nosopsyllus iranus theodori* são maiores e percorrem distâncias maiores em seus pulos do que os machos (Krasnov *et al.*, 2003).

3. Moscas das frutas como modelo para estudos de fisiologia térmica

Os diversos impactos das mudanças climáticas sobre a fisiologia dos organismos podem ganhar um importante aspecto de aplicação econômica quando associados ao risco do aumento da distribuição e incidência de espécies-praga, por

exemplo (Meynard *et al.*, 2013). Este risco para insetos está associado à ação da temperatura sobre o desenvolvimento, fecundidade, extensão do período propício para o desenvolvimento desses animais, dispersão (Bale *et al.,* 2002) e mudanças de relações interespecíficas (Dukes *et al.*, 2009). Insetos tropicais podem vir a ocupar altitudes e latitudes maiores, além de infestar rapidamente novos locais (Fand *et al.*, 2012). Tal dispersão é especialmente relevante para insetos-praga, dada seu grande impacto no setor agropecuário. Assim, a compreensão de fatores ambientais, como a temperatura, sobre esses insetos tem grande relevância para países que dependem da produção e exportação de produtos agrícolas, bem como países que dependem da importação destes produtos para seu abastecimento.

Moscas do gênero *Anastrepha*, juntamente com a espécie *Ceratitis capitata*, são as principais pragas da fruticultura brasileira (Zucchi, 2000). Dentre elas, a espécie de mosca-das-frutas Sul Americana *Anastrepha fraterculus* (Wiedemann) destaca-se por sua polifagia, com 116 espécies de hospedeiras associadas, (Zucchi & Moraes, 2008) e ampla distribuição geográfica, que abrange desde a América Sul até a América Central (CABI, 2018). Moscas *Anastrepha sp.* desempenham comportamentos elaborados durante a corte e oviposição (Aluja *et al.*, 1999). Após a cópula, fêmeas de *A. fraterculus* buscam e examinam frutos, quando encontram um fruto apropriado inserem seu acúleo, depositam seus ovos e marcam o fruto hospedeiro (Aluja *et al.*, 1999). Essa perfuração da epiderme do fruto gera deformações no local (Salles, 1995) e permite a entrada de patógenos (Santos *et al.*, 2008). Adicionalmente, o desenvolvimento dos três primeiros instares larvais ocorre dentro do fruto, favorecendo o apodrecimento do mesmo (Salles, 1995) e inviabilizando seu comércio. Além disso, *A. fraterculus* pertence a um complexo de espécies crípticas (complexo *Anastrepha fraterculus*), no qual duas ou mais espécies são erroneamente agrupadas em uma única nomenclatura (Bickford *et al.*, 2006). Esse complexo conta com três espécies no Brasil (Selivon *et al.*, 2004; Prezotto et al., 2019), as quais foram caracterizadas através da análise intergrada de distintas características biológicas, tais como isozimas, cariótipos, morfometria e morfologia dos ovos (Selivon & Perondin, 1998; Selivon *et al.*, 2004). Essa dificuldade em distinguir as espécies do complexo acaba comprometendo o controle dessa praga.

No Brasil, as medidas de controle populacional de *A. fraterculus* baseiam-se no uso de parasitas nativos, uso de iscas tóxicas e ensacamento de frutos (Nava &

Botton, 2010). Dentre vários aspectos, um dos que podem contribuir para o controle biológico do grupo é como a temperatura afeta determinantes do valor adaptativo dessa espécie. Sabe-se que a temperatura influencia o potencial reprodutivo (Carodoso *et al.,* 2002), longevidade (Cardoso *et al.,* 2002; Taufer *et al*., 2000) e a maturação ovariana (Taufer *et al*., 2000) de *A. fraterculus*. Assim, nota-se que a temperatura é determinante para diversos aspectos da história de vida de espécie do complexo *A. fraterculus*. Entretanto, sabe-se pouco sobre o efeito dela no desempenho locomotor de adultos, o qual pode ser determinante para a capacidade de fuga predadores, bem como para o desempenho de comportamentos elaborados durante a corte e de oviposição. Além disso, a compreensão da sensibilidade térmica do desempenho locomotor e *A. sp.1 aff. fraterculus* pode fornecer novas informações para trabalhos futuros sobre a distribuição da espécie em decorrência das mudanças climáticas, melhoria de técnicas para o controle de pragas e estudos de evolução da fisiologia térmica das moscas do complexo *Anastrepha fraterculus.*

Considerando o efeito da temperatura sobre o desempenho locomotor essa dissertação teve como primeiro objetivo determinar a sensibilidade térmica desse desempenho locomotor forçado e voluntário, bem como a tolerância térmica da espécie *Anastrepha sp1. aff fraterculus,* do complexo *Anastrepha fraterculus*. Nosso segundo objetivo foi verificar a sensibilidade térmica de uma enzima limitante do metabolismo aeróbico (CS) e sua relação com a sensibilidade térmica no desempenho locomotor. Por fim, nosso terceiro objetivo foi testar a existência de compromissos entre os parâmetros da *TPC V_{max}* e *T_{br}* em uma população de *Anastrepha sp1. aff fraterculus*, como proposto pela hipótese "*Jack-of-all temperatures is a master of none".*

Esta dissertação de mestrado é composta por um capítulo na forma de artigo científico. Tal artigo contempla os três objetivos da dissertação e conta com: *Abstract, Introduction, Material and Methods, Results, Discussion, References, and Appendices*. Ao final, a dissertação contará com uma consideração final sobre o tema.

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Artigo

Thermal sensitivity and thermal tolerance of *Anastrepha sp.1 aff. fraterculus* (Diptera, Tephritidae)

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Abstract

Environmental temperature is particularly relevant to small ectotherms, such as insects, given their vulnerability to abrupt changes in body temperature. These changes can impact performances highly relevant for fitness, like locomotor performance. Here, we investigated the thermal limits and thermal sensitivity of locomotor performance and aerobic capacity of a South American fruit fly pest, *Anastrepha sp.1 affinis fraterculus*. We measured flies walking speed and voluntary locomotion at five temperatures (13, 18, 23, 28 and 33°C). We measured flies critical thermal minimum (CT_{min}) and maximum (CT_{max}) by increasing and decreasing environmental temperature, respectively, with a rate of 1.0°C.min^{-1}. At the cellular level, we assessed citrate synthase activity at four temperatures (13, 23, 33 and 43°C). Our results showed a left-skewed inverted U-shaped thermal sensitivity curve for forced locomotor performance, with optimal temperature ~28.7°C, CT_{min} ~ 3.9 $^\circ$C and CT_{max} ~ 43.1 $^\circ$C. Flies had low frequency of voluntary locomotion, but they walked more frequently at intermediate temperatures (18 to 23°C). Males were more tolerant to heat than females, and larger individuals where more cold tolerant. Citrate synthase activity increased with temperature up to 43°C, which was closer to males' CT_{max} and higher than females' CT_{max}. Thus, thermal sensitivity of total mitochondrial aerobic capacity does not represent an important isolated determinant of thermal sensitivity of locomotor performance for this species. The lack of correlation between maximum speed and thermal performance breadth lead us to reject the hypothesis of '*jack-of-all-temperatures is a master of none*' for *A. sp.1 aff. fraterculus* locomotor performance.

Keywords

1. Introduction

The effect of temperature has been documented on nearly all levels of animal organization, from molecular (Angilletta *et al.,* 2009; Hochachka and Somero, 2002; Somero, 1995) to ecological interactions (Bale *et al*., 2002). Indeed, thermal sensitivity at organismal level might be a result of temperature effects across multiple levels of biological organization (Chaui-Berlink *et al.*, 2004). Fluctuations of environmental temperature are particularly relevant to small ectotherms, such as insects, given their low thermal inertia and vulnerability to abrupt changes in body temperature (Kemp and Krockenberger, 2004; McCue and De Los Santos, 2013). As a matter of fact, changes in environmental temperature can modulate foraging activity of insects (Jayatilaka *et al.*, 2011), life history (Ma *et al*., 2017; Uyi *et al.*, 2016), metabolic rate (Berrigan and Partridge, 1997; Nespolo *et al.*, 2003) and locomotor performance (Cavieres *et al.*, 2016; Dillon, 2006).

Typically, the relationship between body temperature and multiple biological performances of ectotherms are described by thermal performance curves (*TPC*) (see Schulte *et al.,* 2011). In general, *TPCs* are depicted by an inverted U-shape pattern within a thermal tolerance range. Many biologically meaningful parameters can be estimated from *TPCs*, such as: the temperature in which performance is maximized (V_{max}), the optimal temperature (T_o); critical thermal minimum (CT_{min}); critical thermal maximum (CT_{max}) and thermal performance breadth ($T_{br}=CT_{max}-CT_{min}$). Several studies have highlighted the practical relevance of estimating thermal limits in order to determine potential organisms' geographic distribution and responses to global climate change (Andersen *et al.*, 2015; Deutsch *et al*., 2008*;* García-Robledo *et al.*, 2016; Overgaard *et al.*, 2014). Moreover, *TPCs* allow tests of multiple hypotheses involving possible trade-offs between thermal parameters. One fundamental question in thermal physiology regards the existence of a trade-off between maximum performance (P_{max}) and T_{br}, known as the '*jack-of-all-temperatures is a master of none*' hypothesis (Huey

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[1]*TPC*: Thermal performance curve, CT_{min}: critical thermal minimum, CT_{max}: critical thermal maximum, T_{br}: thermal performance breadth, V_{max}: maximum speed, T_o: optimal temperature*, P_{max}*: maximum performance*,* CS: citrate synthase*, B_{80}*: performance breadth of 80% maximum performance.

and Hertz, 1984). An alternative hypothesis, called '*jack–of–all-temperatures is a master of all*' lead to prediction that individuals that perform better at T_o have broader T_{br} (Huey and Hertz, 1984). Even though the trade-off between P_{max} and T_{br} is predicted by theory (Gilchrist, 1995), empirical studies have reported different patterns of relation between these variables (Huey and Hertz, 1984; Palaima and Spitze, 2004).

The effects of temperature on locomotor performance have been considered of high relevance to fitness. Locomotor performance can be associated with the organismal capacity to perform paramount behavioral traits, such as searching for food and mating partners, and avoiding predation (Higham, 2007; Kasumovic and Seebacher, 2018; and Pruitt and Troupe, 2010). According to the morphology-performance-fitness paradigm, natural selection acts on organisms' behavior and performance, which are determined by subjacent morphological and physiological traits (Arnold, 1983; Garland and Carter, 1994). Accordingly, organisms' behavior and performance would show higher and more direct impact on fitness than their subjacent determinants (Arnold, 1983; Garland and Carter, 1994). Arthropod locomotor performance, for example, is relevant to several determinants of fitness including mating success (e.g., *Phoracantha semipunctata*, Hanks *et al.*, 1996; *Libellula pulchella*, Marden and Cobb, 2004; and *Drosophila* species, Partridge *et al.,* 1987), and strategies to escape from predators (e.g., wolf spiders, Nelson and Formanowicz, 2005). Given that insects in general depend essentially on aerobic pathways to supply the energy demand for locomotor activities (Full and Tullis, 1990; Suarez *et al.*, 1996, 1998, 2000), thermal sensitivity of aerobic metabolism might limit and shape their locomotor *TPC*. Furthermore, body size variation affects several physiological and ecological performances, as well as behaviors, in insects (Hanks, *et al.*, 1996; Honěk, 1993; Hozumi, 2005; Niven and Scharlemann, 2005). Larger insects, for example, are less susceptible to viscous surface forces (*e.g.* electrostatic attraction), but are more susceptible to gravity forces than smaller individuals (Whitman, 2008). Additionally, body size is also a common sexual dimorphic trait for many arthropods and has been related to sex differences in locomotor performance of some species (Carlson, *et al.*, 2014; Gilchrist, 1996; Krasnov *et al.*, 2003).

Tephritidae fruit flies are the main pests of Brazilian fruticulture (Zucchi, 2000). Among them, the South American fruit fly species *Anastrepha fraterculus* (Wiedemann) stands out, given its polyphagia (Zucchi and Moraes, 2008) and wide geographic distribution, occurring from South America to Central America (CABI,

2019). Females from this species are typically larger than males (Salles, 1995) and previous studies demonstrated that temperature is an important trait for *A. fratercutlus* life history. For instance, *A. fraterculus* development is interrupted at temperatures lower than 10.7$^{\mathrm{o}}$C (Machado *et al*., 1995), ovarian maturity occurs only from 20.0 to 25.0$^{\mathrm{o}}$C (Taufer *et al*., 2000), and females of *A. fraterculus* have higher longevity at 13.0$^{\mathrm{o}}$C (Taufer *et al.*, 2000). Moreover, flies of *A. fraterculus* from both sexes reared under constant cold regime (6.0$^{\mathrm{o}}$C) and alternating temperature regimes (20.0/6.0$^{\mathrm{o}}$C and 25.0/13.0$^{\mathrm{o}}$C) had higher life spam and life expectancy when compared to individuals reared in a constant regime of 25.0$^{\mathrm{o}}$C (Cardoso *et al.,* 2002*)*. However, the effects of temperature on locomotor performance of these flies remains unexplored.

Here, we aimed to describe thermal limits and thermal sensitivity curve (*TPC*) of forced locomotion of *Anastrepha sp.1 affinis fraterculus,* one species belonging to the *fraterculus* complex (Hernández-Ortiz *et al*., 2015; Prezotto *et al.*, 2019). We predicted that forced locomotor *TPC* would fit an inverted U-shape curve. Considering that females are usually larger than males, we predicted that females are faster than males in all temperatures tested. We also investigated the influence of temperature on voluntary locomotion and frequency of manifestation of different behavioral traits in *Anastrepha sp.1 aff. fraterculus*. We predicted that manifestation of different behaviors, including voluntary locomotion, would increase at temperatures that maximize forced locomotor performance. Moreover, we aimed to assess thermal sensitivity of an important catalytic step determinant of the aerobic capacity (whole body citrate synthase activity, CS). We hypothesized that flies' aerobic capacity would be an important subjacent trait determining the thermal sensitivity of forced locomotor performance. Accordingly, we predicted similar patterns of *TPC* for CS activity and forced locomotor performance. We also predicted that females would have higher aerobic activity (higher CS activity at different temperatures) than males. Finally, we tested the hypothesis *'jack-of-all-temperatures is a master of none'*, and predicted that there is a trade-off between V_{max} and T_{br} values.

2. Material and Methods

Experimental model system

Individuals of *Anastrepha sp.1 aff. fraterculus* were obtained from a colony maintained in laboratory for about 10 generations at 25.0±0.3$^{\mathrm{o}}$C and 12:12hrs of light:

dark cycle. This fruit fly colony was derived from infested loquats (*Eriobotrya japonica Lindl*) collected in Agronomic Institute of Campinas, municipality of Jundiaí, SP, Brazil (22°53'28.0"S, 47°03'53.2"W). From this colony, 100 pupae were recovered in June 2018 (sample A) and 200 pupae in June 2019 (sample B). The pupae were covered with moistened vermiculite to avoid dehydration and maintained in a small plastic container (width: 13cm, length: 23cm, height: 13cm). This plastic container was kept under controlled temperature of $25.0 \pm 0.3^{o}C$ and 12:12hrs of light: dark cycle, inside a climatic chamber (BOD EL212/3–Eletrolab, São Paulo, SP, Brazil). In order to prevent experimental use of adults of very different ages, only those emerged within a period of three days were included in the assays. Virgin adult flies were separated by sex to avoid matting and consequently differences in sperm-storage organs among females (Fritz, 2004). Individuals were submitted to the tests 11 days after adult emergence, when flies were reproductively mature (Lima and Howse, 1997) in order to reduce behavioral differences due to mature stage (Lima and Howse, 1997).

One day before the first test, individuals were marked on their thorax and abdomen with a permanent non-toxic pen (Sharpie Inc.) combining the colors: red, green, blue, purple, black and gold, and the body masses of flies were measured using a semi analytical balance ($\pm$ 0.0001g) (BEL engineering, Monza, MB, Italy). Individuals of sample A (N=17) were used for measurements of aerobic capacity (whole body citrate synthase activity). Given that 14 of 40 flies died after being marked with the non-toxic pen in 2018 we changed the marking tool in the following year. Thus, flies from sample B (N=61) received marks on thorax and abdomen with gouache paint combining the colors: red, white, yellow, black, blue and gray. These flies were used for measurements of forced locomotor performance, voluntary locomotion and thermal limits.

Locomotor performance at different temperatures

To investigate the temperature effects on locomotor performance, walking speed ($cm.s^{-1}$) of flies submitted to five thermal treatments (13, 18, 23, 28 and $33 \pm 1^{o}C$) was measured, in a random sampling order. The essays were performed inside a walk-in chamber with temperature and humidity control (FITOTRON-Eletrolab, São Paulo, SP, Brazil). Since two flies died when exposed to $33^{o}C$, this temperature was established as the highest thermal treatment. Flies body temperature

was assessed with a T-type thermocouple (<0.4mm diameter, Physitemp Instruments, Inc., Clifton, NJ, USA) inserted in the thorax of a dead fly exposed to the same environmental conditions as the live flies. Flies were kept at $25^{o}C$ between experimental procedures to avoid acclimation effects.

The locomotor performance was measured according to Gilchrist's (1996) methodology with slight modifications. Briefly, flies were randomly grouped in 8 to 10 individuals prior to tests. The flies were kept inside the environmental chamber for 10 minutes to achieve thermal equilibrium. To measure walking speed, each fly was placed in a sealed vertical polypropylene plastic tube (diameter: 1.5 cm; length: 20.0 cm) and the time spent to walk 17.0 cm up was registered. A bamboo stick (length: 24.0 cm) was employed to gently encourage the flies to walk in a nonstop way through the plastic tube. The tests were conducted between 8h00 to 17h00 and repeated twice for each individual. The walking speed of flies at each temperature was determined by the average time of the two trials.

Voluntary locomotion, stimulated and non-stimulated behavior

To assess the temperature effects on voluntary locomotion, 40 randomly sampling flies (20 females and 20 males from sample B) were analyzed at the temperatures 13, 18, 23, 28 and 33 ± 1 ^{o}C. At the end of forced locomotor performance test, each fly was placed inside an upside down transparent glass bottle (500ml) and its behavior was recorded with a smartphone device (iPhone SE – Apple, Cupertino, CA, USA) for five minutes. Flies body temperature were assessed with a T-type thermocouple (<0.4mm diameter, Physitemp Instruments, Inc., Clifton, NJ, USA) inserted in the thorax of a dead fly placed inside another upside down glass bottle as reference of the body temperature of live flies.

All videos (N=200) were analyzed by a single person to avoid bias interpretation among observers. Behaviors were scored using the free behavior observation software *Boris* (Friard and Gamba, 2016). The ethogram used for behavior analyses included two behavioral categories: State behaviors and point events. State behaviors, standardized as behaviors lasting longer than 0.5s and mutually exclusive included: (i) walking: locomotion on a substrate following a defined direction; (ii) flying: aerial locomotion by flapping their wings; and (iii) stationary: the animal remain at the same place. Point events, standardized as small and rapid behaviors that happened while flies were stationary included: (i) body

adjustment: changes in body direction or position; and (ii) cleaning events: cleaning it wings and head with legs.

After five minutes of the stimulus-free behaviors records, flies were stimulated with ten standardized knocks with a bamboo stick (diameter: 4mm, length: 24 cm) on the bottle glass external surface. Flies' reactions to stimulus were accounted as the number of times they flew in response to the knocks.

Lower and upper critical temperatures

Both, CT_{min} and CT_{max} were assessed using a dynamic method with gradual cooling and heating with rates of $1.0^oC.min^{-1}$ and accuracy of $±0.1^oC$. The CT_{min} measurements were taken 48h after the end of locomotor performance tests, and CT_{max} was assessed 48h after CT_{min} measurements.

For critical thermal limits assays, the flies were sorted into groups of 5 to 6 individuals. Each individual was placed inside a falcon tube (50ml) with three lateral small holes to ensure gas exchange and avoid hypoxia. To assess flies body temperature, one dead fly with T-type thermocouple inside its thorax was placed in one falcon tube (50ml) and submitted to the same experimental condition as the live flies. A programed environmental chamber (SH - 661 Benchtop Chamber, ESPEC Tenjinbashi, Kita-ku, Osaka, Japan) was used to promote the gradual and constant rate of change in environmental temperature. First, flies were set inside the environmental chamber at 25^oC for 15 minutes to ensure all flies started the tests with the same body temperature (Terblanche *et al.*, 2007). Thereafter, the temperature inside the chamber decreased or increased at constant rate for CT_{min} and CT_{max}, respectively.

As the temperature decreased in CT_{min} tests, flies reduced their walking activity until they fell to the falcon flasks bottom, contracted their locomotors appendices and entered in a reversible paralysis and state of chill coma. At this point, the temperature registered by the T-type thermocouple inside the dead fly was defined as CT_{min}, in accordance with previous studies by MacMillan and Sinclair (2011); Hazell and Bale (2011), and Schou *et al.* (2017). When the last fly achieved its CT_{min}, flies were removed from the environmental chamber, and let recover at 25^oC for 48 hours before CT_{max} measurement. During CT_{max} measurements, flies increased their walking activity until lost their muscle control (Lutterschmidt and Hutchison, 1997). At this temperature, they fell to falcon bottom, contracted their locomotors appendices

and were incapable of walking. The temperature registered by the T-type thermocouple inside the dead fly was defined as their CT_{max}. After the last fly achieved its CT_{max}, we removed the flies from chamber, and let all recover for 24 hours at 25^oC.

Aerobic activity in different temperatures

The aerobic activity was estimated using the activity of the enzyme citrate synthase (CS) of individuals from sample A. First the flies were anesthetized in the ice and then had their wings removed with a scalpel. Next, were euthanized in liquid nitrogen and their bodies (head, thorax, appendices and abdomen) stored in -80^oC freezer.

Each crude extract sample consisted of one fly's body homogenized in buffer solution (1:10) with a Turrax homogenizer (Ultra 80, Ultra Stirrer Inc.) immersed in an ice bottle. The buffer solution was composed by Imidazol-HCl-20mM (pH 7.4) with a protease inhibitor phenylmethanesulfonyl fluoride 1mM (PMSF), a calcium-chelating agent 2mM-EDTA and a phosphatase inhibitor sodium fluoride 20mM-NaF and detergent solution 1% -Triton-X100. The homogenates were submitted to sonication in ice with a Microson sonicator (XL200, Misonix Inc., Farmingdale, NY, USA) to disrupt the phospholipid cell and mitochondrial membranes. The sonication in ice was performed to avoid heating, with 3 pulses of 25kHz for 10 seconds and interval of 10 seconds between pulses.

CS activity was quantified with a spectrophotometer (DU 800 UV/Vis-Beckman Coulter Inc. Fullerton, CA, USA), in non-inhibitory substrates condition, according to Bergmeyer (1983). The absorbance of dithio-nitrobenzoic acid (DTNB) of the control was compared with reaction assays to determinate CS activity. The control assay was composed by 522μl of Tris-HCl 50 mM (pH 8.0), 70μl of DTNB 1mM, 50μl of Acetil-CoA 3mM and 28μl of fly homogenate (diluted 1:20). The reaction assay included all the reagents used on the control assay, and 30μl of oxaloacetate 20mM. Both assays were incubated for 3 minutes at each thermal treatment (13, 23, 33 and $43^oC\pm1 \ ^oC$) and the absorption was read at 420mm. CS activity was calculated by the formula:

$$EA = [(\Delta Abs.min^{-1}.total \ dilution)]/(\varepsilon.d)$$

Where *EA* is the CS activity at assay conditions, $\Delta Ab.min^{-1}$ is the absorbance variation per minute, calculated as the difference between the absorbance of reaction

assay and control assay, total dilution is the sample total dilution, ε is the DTNB coefficient of millimolar ($13.6.10^2.mol^{-1}.mm^{-1}$) and d is the distance that light traveled through the sample (10mm). The results were expressed in μmol of substrate converted in product per minute per gram of fresh tissue ($U.min^{-1}.g^{-1}$).

Statistical Analyses

Statistical analyses were performed in R software (R Core team: 2018, version 3.5.1). Descriptive statistics were performed for variables related to performance (walking speed, voluntary locomotion, stimulated behavior and CS activity) by gender and temperature; and variables related to flies body size and thermal limits (body mass, CT_{min} and CT_{max}) by gender. In order to explore the effect of temperature on walking speed, CT_{min} and CT_{max} values were included as temperatures in which the animal speed was zero. Then, a TPC for each individual was constructed comparing a set of parabolic equations (Gaussian, Lorentzian, Logistic, Exponential Modified Gaussian, Gamma, Weibul, Modified Gaussian and Quadratic) built into the statistical program TableCurve2D (Angilletta, 2006, Gallo *et al*., 2020; Logan *et al*., 2015). The most frequent equation with highest F-statistic was chosen as the best model. Thereafter, the selected equation was applied for each individual and the TPC parameters: T_o, V_{max}, and temperature breadth at which performance values exceed 80% of the maximum performance (B_{80}) were extracted.

To access the effect of gender and body mass on flies' TPC parameters, general linear mixed models with the packages bbmle and nlme in R software were performed. The models included the following variable: CT_{min}, CT_{max} T_{br}, V_{max}, T_o and B_{80} as dependent variables, body mass and sex as predictors, and individuals were included as random factors. Models were selected using the principle of parsimony, comparing Akaike's information criterion corrected for small samples (AICc) of mixed models. All models with AICc values lower than 2.0 were considered valid models and the most parsimonious had higher weight (Burnham and Anderson, 2002; Symonds and Moussalli, 2011).

Regarding voluntary behavior, the proportion of time each individual spent performing a state event behavior (walking, flying and stationary) and the number of times they performed a point event behavior (body adjustment and cleaning events) were calculated. Thereafter, general linear mixed models with these behaviors as dependent variables, body mass and sex added as predictors, and individuals included

as random factors were modeled. Again, the models were selected using the principle of parsimony AICc as previously described.

To test the effect of temperature, sex and body mass on CS activity, first the polynomial function (first, second or third order) that best fitted our data was chosen comparing general linear mixed models. Subsequently other set of general linear mixed models with CS activity as dependent variable, sex and body mass inserted as predictor variables and individual identity included as random factor we tested. The models with AICc <2.0 were considered valid while the model with higher weight was the most parsimonious.

Finally, to test the '*jack-of-all-temperature is a master of none*' hypothesis a normality test Shapiro-Wilk was conducted for V_{max} and T_{br}. Since the variables did not have normal distribution, the non-parametric test of Kendall's coefficient of concordance was applied to assess the relationship between V_{max} and T_{br}.

3. Results

Thermal sensitivity of forced locomotion

The descriptive statistics of flies body mass, walking speed, and *TPC* parameters are shown in Appendix (Table A.1). The *TPC* of 58 flies were described by EMG equation (Figure 1). The *TPC* rising phase of females occurred from $3.9\pm0.9^{o}C$ (CT_{min}) up to $28.8\pm3.4^{o}C$ (To), followed by a prominent decrease of locomotor performance until $43.0\pm0.6^{o}C$ (CT_{max}). Males rising phase occurred from $4.1\pm0.7^{o}C$ (CT_{min}) up to $28.7\pm3.1^{o}C$ (T_{o}), and then decreased until $43.3\pm0.5^{o}C$ (CT_{max}).
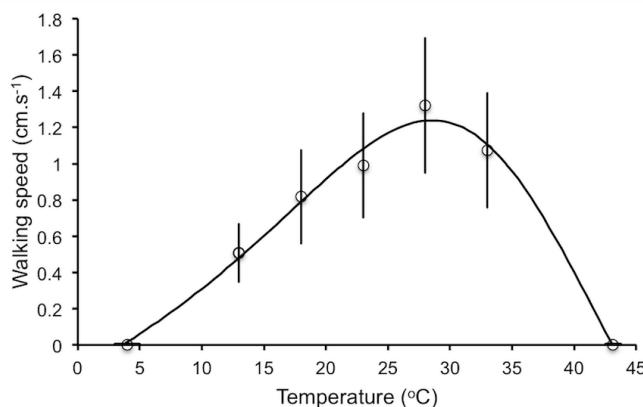

Figure 1. Thermal performance curve of *Anastrepha sp.1 aff. fraterculus* (N=58). Circles represent mean values with standard deviation lines.

Table 1 shows all plausible models from mixed model tests. The models for CT_{min}, V_{max}, T_o, T_{br} and B_{80} might not influenced by influenced by body mass or sex, given that the null model is equally plausible. The model with higher weight for CT_{min} indicated that larger individuals were more tolerant to cold, independently from gender (Figure 2). The third one indicated that larger individuals and males were more tolerant to cold. For CT_{max} the models indicated that males were more tolerant to high temperatures than females, and the other plausible models for CT_{max} indicated that smaller individuals and males were more tolerant to higher temperatures.

Table 1. Mixed models test the effect of sex and body mass on the operative variables obtained from the thermal performance curve with AICc values <2.0

Dependent	Predictor	Random factor	AICc	dAICc	df	Weight
CT_{min}	Body Mass	ID	144.5	0.0	4	0.41**
	Null	ID	145.7	1.2	3	0.22
	Sex+ Body Mass	ID	146.4	1.8	5	0.16
CT_{max}	Sex	ID	102.9	0.0	4	0.48**
	Sex+ Body Mass	ID	104.6	1.7	5	0.21
T_{br}	Null	ID	177.6	0.0	3	0.38**
	Body Mass	ID	178.2	0.6	4	0.28
	Sex	ID	179.6	1.9	4	0.15
V_{max}	Null	ID	78.5	0.0	3	0.43**
	Sex	ID	79.5	1.0	4	0.26
T_o	Null	ID	306.1	0.0	3	0.51**
	Body Mass	ID	308.0	1.9	4	0.20
B_{80}	Null	ID	327.3	0.0	3	0.44**
	Body Mass	ID	328.4	1.1	4	0.25

AICc: Akaike information criterion corrected for small sample sizes; **dAICc**: delta AICc; **df**: degrees of freedom; **Weight**: weight of AICc; V_{max}: flies' maximum walking speed; T_o: optimum temperate; B_{80}: temperature at which performance values exceed 80% of the maximum performance; CT_{min}: minimum critical temperature; CT_{max}: maximum critical temperature. **Null**: model with no effect of predictor variables; **Sex**: females and males as categorical variable; **Body mass**: flies weight as continuous variable; **ID**: individual as random factor; **(+)**: additive effect of variables; **(**)**: model with higher weight.
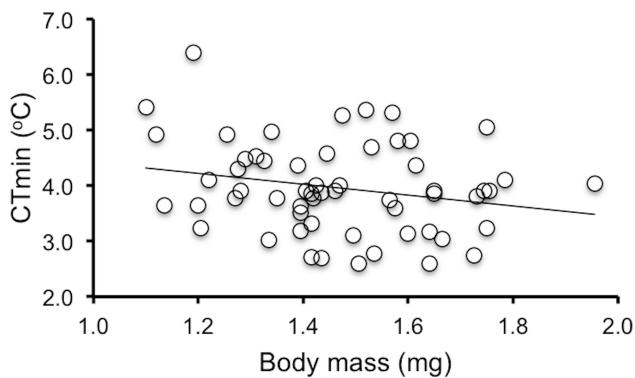
Figure 2. Relationship between minimum critical temperature (CT_{min}) and body mass of flies *Anastrepha sp.1 aff. fraterculus* (N=58)

Other plausible models indicated that body mass might influence T_{br}, T_o and B_{80}; in that case, larger flies had broader temperature ranges (higher T_{br} values). Smaller individuals had higher T_o and achieved 80% of their maximum velocity in a broader temperature range than larger individuals. The other selected model for V_{max} indicated that females were faster than males.

Voluntary locomotion and stimulated behavior

Descriptive statistics of scored behaviors for females and males of *A. sp.1 aff. fraterculus* are shown in Appendix (Table A.2). Table 2 shows all plausible models from mixed model tests. Males spent more time walking in intermediate temperatures (18 to 28°C) than females, while flies with intermediate size were more willing to walk (Figure 3.A). Given that the null model was selected there might be no effect of temperature, body mass or sex on the proportion of time flies spent walking. Moreover, smaller flies flew more frequently (Figure 3.B) and remained less stationary at higher temperatures (18 to 33°C) than larger flies (Figure 3.C).

Table 2. Mixed models to test the effect of temperature, body mass and sex on flies A*nastrepha sp.1 aff. fraterculus* voluntary locomotion and flying response with AICc<2.0

Dependent	Predictor	Random factor	AICc	dAICc	df	Weight
Walking	Temperature *Sex*Body mass	ID	-342.0	0.0	10	0.23**
	Null	ID	-340.4	1.6	3	0.10
	Temperature	ID	-340.5	1.6	4	0.10
	Temperature+ Body mass	ID	-340.3	1.7	5	0.10
	Body mass	ID	-340.3	1.7	4	0.09

	Temperature* Body mass	ID	-340.2	1.8	6	0.09
Fly	Temperature* Body mass	ID	-1428.6	0.0	6	0.42**
Stationary	Temperature *Body mass	ID	-269.0	0.0	6	0.40**
	Temperature + Body mass	ID	-267.5	1.5	5	0.19
Body adjustment	Temperature	ID	1356.4	0.0	4	0.21**
	Temperature+ Body mass	ID	1356.5	0.1	5	0.20
	Temperature +Sex	ID	1356.9	0.5	5	0.16
	Temperature*Body mass	ID	1358.2	1.8	6	0.09
	Temperature +Sex +Body mass	ID	1357.9	1.6	6	0.09
Cleaning	Temperature*Sex	ID	1409.4	0.0	6	0.80**
Flying response	Null model	ID	-726.9	0.0	3	0.23**
	Sex	ID	-726.1	0.8	4	0.15
	Temperature	ID	-726.0	0.9	4	0.14
	Temperature+Sex	ID	-725.2	1.7	5	0.10

AICc: Akaike information criterion corrected for small sample sizes; **dAICc**: delta AICc **df**: degrees of freedom; **Weight**: weight of AICc; **Walking**: proportion of time spent walking; **Stationary**: proportion of time spent stationary; **Body adjustment:** number of times flies changed their position while stationary; **Cleaning**: number of times flies cleaned their wings and head while stationary; **Flying response**: proportional number of times flies flee in response to ten hits on the bottle; **Null**: model with no effect of predictor variables; **Temperature**: assay temperatures (13,18, 23, 28 e 33°C) as fixed variable; **Sex**: females and males as predictor variables; **ID**: individual as random factor; (+): absence of interaction between variables; (*): interaction between variables; (**): model with higher weight.
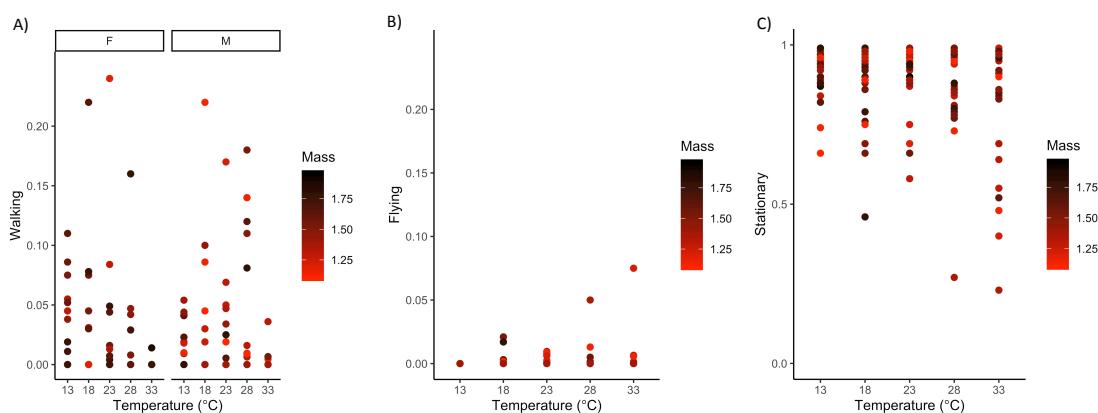
Figure 3. Proportion of time *Anastrepha sp.1 aff. fraterculus* (N=40) spent performing state event behaviors at different temperatures. (A) Proportion of time spent walking as a function of body temperature (^{o}C), body mass (mg) and gender. Data set on the left (F) is the proportion of time females spent walking and the data set on the right (M) is the proportion of time males spent walking. (B) Proportion of time flies spent on flying as a function of body temperature and body mass (mg). (C) Proportion of time flies spent on stationary condition as a function of body temperature and body mass (mg). Lighter flies are in red and the heavier are in black following the color gradient (red-black) in 3.A, 3.B and 3.C.

The models showed that body adjustments were more frequent at higher temperatures (Figure 4.A). There is a possible additive effect of temperature and body mass, in which smaller individuals also changed their body position more frequently. Females and males increased the frequency of cleaning behavior by 4.3 fold and 2.6 fold, respectively, from 13°C to 33°C (Figure 4.B).
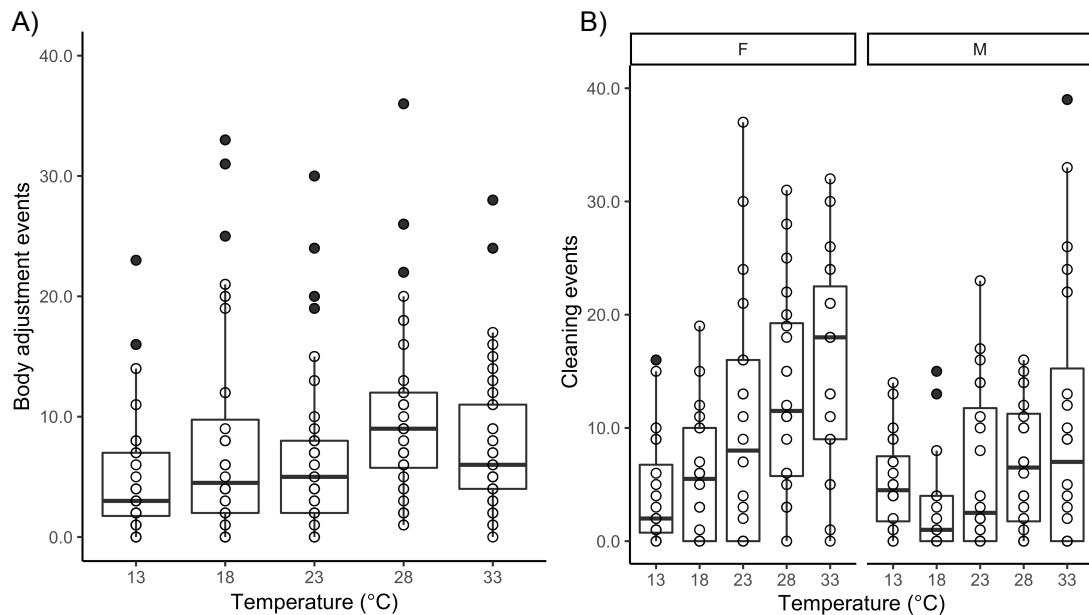
Figure 4. Occurrence of point event behaviors in A*nastrepha sp.1 aff. fraterculus* (N=40) at different temperatures. (A) Body adjustments. (B) Cleaning behaviors. The boxplots on the left (F) are females' cleaning events and boxplots on the right (M) are males' cleaning events. The boxplots show the lower (Q1) and upper (Q3) quartile range of data, line in the middle is the median and the vertical lines are the standard deviation. Most individual data values are represented as empty circles, while outliers are represented as filled circles.

The plausible models for *A. sp.1 aff. fraterculus* flying response to stimulus showed that the null model is equally plausible in comparison to others (Table 2) indicating the absence of temperature, sex an body mass on flying response. The second plausible model for this variable indicates that males flew more frequently than females in response to stimulus. The third model indicates the effect of temperature on this behavior, in which there was no flying response scored at 13°C for both sexes, while the fourth model indicated an additive effect of temperature and gender.

Thermal sensitivity of aerobic pathway

Descriptive statistics of CS activity for females and males of *A. sp.1 aff. fraterculus* are available in the Appendix (Table A.3). The plausible models indicate that citrate synthase activity increased with increasing assay temperature (Table 3). The most parsimonious model showed an influence of gender and body mass on CS activity (Table 4). Females were larger and had higher CS activity than males (Figure 5).

Table 3. Models tested to determinate functions that best fit the thermal sensibility of *Anastrepha sp.1 aff. fraterculus* citrate synthase activity

Model	AICc	dAICc	df	Weight
Linear	593.7	0	3	0.60**
Quadratic	595.2	1.5	4	0.29

AICc: Akaike information criterion corrected for small sample sizes; **dAICc**: delta AICc; **df**: degrees of freedom; **Weight**: weight of AICc; **Null**: model with no effect of independent variable; **Linear**: model that fit a linear regression to data; **Quadratic**: model that fit a quadratic expression to data; **(**)**: model with higher weight.

Table 4. Mixed models to test the effects of temperature, sex and body mass on citrate synthase activity of flies *Anastrepha sp.1 aff. fraterculus*

Dependent	Predictor	Random factor	AICc	dAIC	df	Weight
CS	Temperature*Sex*Body mass	ID	546.4	0	10	0.29**
CS	Temperature	ID	546.9	0.5	4	0.22
CS	Temperature + Sex	ID	547.6	1.2	5	0.16
CS	Temperature* Sex	ID	547.7	1.2	6	0.15

AICc: Akaike information criterion corrected for small sample sizes; **dAICc:** delta AICc; **df**: degrees of freedom; **Weight**: weight of AICc; **Null**: model with no effect of fixed variables; **CS**: citrate sintase activity (umol.min^{-1}.g tissue^{-1}) as fixed variable; **Temperature**: assay temperatures (13,18, 23, 28 e 33°C) as fixed variable; **Sex**: females and males as fixed variable; **Body mass**: body mass (mg) as fixed variable; **ID**: individual as random factor; **(+)**: absence of interaction between variables; **(*)**: interaction between variables; **(**)**: model with higher weight.
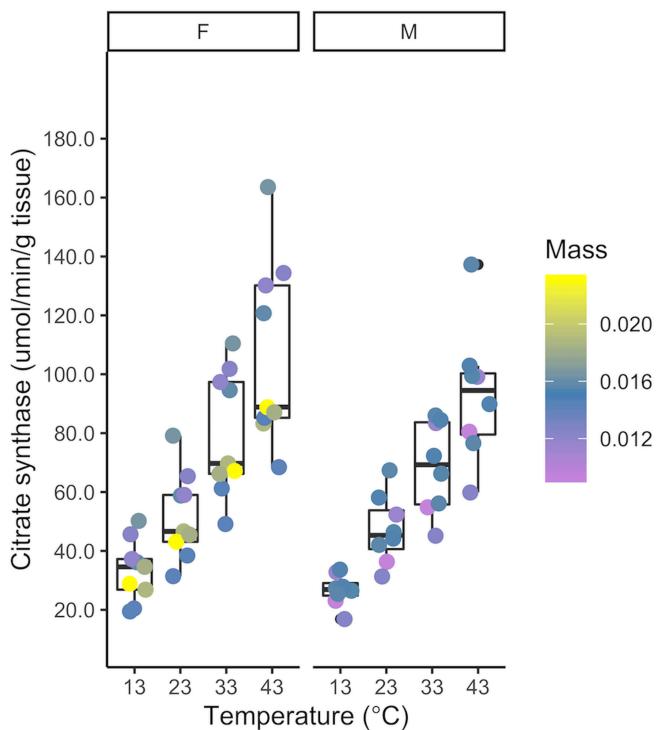
Figure 5. Citrate synthase activity as a function of body temperature (^{o}C), gender and body mass (g) of the flies A*nastrepha sp.1 aff. fraterculus* (N=17) . The boxplots show the lower (Q1) and upper (Q3) quartile range of data, line in the middle is the median and the vertical lines are the standard deviation. The data values are in empty circles and the outliers are the filled circles. The data set on the left (F) represents females while data set on the right (M) represents males. Body mass is indicated by a yellow-blue-violet color gradient in which heavier individuals are in yellow and lighter individuals are in violet.

Maximum speed and thermal performance breadth

Maximum speed is not correlated to thermal performance breadth in *Anastrepha sp.1 aff. fraterculus* flies (Kendall coefficient of concordance =-0.49; *P*=0.59; Figure 6).
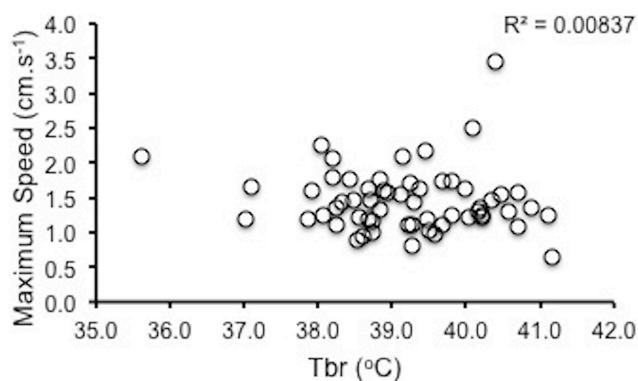
Figure 6. *Anastrepha sp.1 aff. fraterculus* (N=58) maximum speed as a function of thermal performance breadth (T_{br}). Kendall coefficient of concordance =-0.49, *P*=0.59 and R^2 value.

4. Discussion

Thermal sensitivity of forced locomotion

Individuals of *Anastrepha sp.1 aff. fraterculus* exhibited an asymmetrical and left-skewed *TPC* for forced locomotor performance. This *TPC* shape has been reported for locomotor performances on different species of invertebrates (Gilchrist, 1996; Lachenicht et al., 2010; Schuler *et al.,* 2011) and vertebrates (Araspin *et al.,* 2020*;* Ben-Ezra *et al.*, 2008; Hertz *et al,* 1983). However, as far as we know, this study is the first to describe the *TPC* shape of locomotor performance of Tephritidae flies. Usually, the *TPC* rising phase is attributed to thermodynamic effects and the decline of performance is due to protein denaturation (Hochachka and Somero, 2002; Rezende and Bozonovic, 2019; Schute *et al.,* 2011). This same pattern of *TPC* has been described for development rate of different species of Tephritidae group, including the olive fruit fly *Bactrocera oleae* (Wang *et al.,* 2012), immature stages of *Ceratitis rosa* from South Africa and Kenya (Tanga *et al.,* 2015) and different stages of *Bactrocera correcta* (Liu and Ye, 2009). These results suggest that the same thermodynamic principles might be applicable for different biological process and developmental stages.

In our study, the null model was selected as a plausible for the majority of the tested parameters. Thus, both body mass and sex might have no or only a small contribution on *TPC* locomotor performance. Both sexes exhibited a resembling *TPC* shape with similar values of V_{max}, B_{80} and T_{br}, suggesting that gender is not the major trait influencing thermal sensitivity of forced locomotor performance in this species. Otherwise, previous studies have shown *TPC*s are highly influenced by individuals' thermal history. Previous studies reported a variation on *TPC* descriptors between individuals acclimated at distinct temperatures (Esterhuizen *et al*., 2014), and fluctuating thermal regimes (Bozinovic *et al.*, 2013; Cavieres *et al*., 2016; Colinet *et al*., 2015). Since we analyzed individuals from a population maintained at constant temperature for many generations in laboratory conditions, this constant environment might be driving the population to a reduced variability. Therefore, we strongly

encourage further studies with natural (field) populations to investigate possible acclimation influences on *A. sp.1 aff. fraterculus* locomotor performance.

Although sex did not affect locomotor performance, males were slightly more tolerant to high temperatures than females. In previous studies with other Tephritidae flies (C. capitata and C. rosa) they have not found any influence of the genus on thermal tolerance (Nyamukondiwa and Terblanche, 2010). However, this influence was observed in drosophilids: males of *D. melanogaster*, had higher survival rates than females after heat shock treatments (Lansing *et al*., 2000). Previous studies have associated thermal tolerance to high temperatures with expression of heat-shock proteins (Hsp) (Feder *et al*., 1997, Feder, 1999). Both males and females of *Bemisica tabaci*, for example, had higher levels of Hsp70mRNA when exposed to 44°C for 1 hour than individuals exposed to 25°C (Lü and Wan, 2008). However, it is important to note that the role of Hsp expression is controversial, even when gender is not considered. Udaka, *et al.* (2010), for example, proposed that recovery time from heat coma and chill coma have genetic bases, but the expression of Hsp70 was not required for the rapid recovery (30 min at 25°C) from heat coma. Given males of *A. sp.1 aff. fraterculus* were only slightly more tolerant than females to heat, we can not affirm that this species is the best model to explore mechanisms underlying thermal tolerance sexual dimorphism in Tephritidae flies.

Body size rather than gender seems to influence *A. sp.1 aff. fraterculus* cold tolerance. In our study, larger individuals were more tolerant to cold and had a broader thermal tolerance range than the smaller ones. Interspecific studies show that larger insects have a comparatively lower surface-to-volume ratio, and consequently lower rates of heat transfer with environment (Willmer and Unwin, 1981). However, inter-individual differences on body size and its effect on thermal tolerance are poorly explored on insects. In our study, the body mass of *A. sp.1 aff. fraterculus* varied from 1.10 mg to 1.96 mg, a difference of 0.8mg from the smaller individual to the larger one. Our results might suggest that abrupt drops in temperature could end up acting in favor of animals with larger thermal inertia. However, formal tests are necessary to ensure that a difference of only 0.8 mg or less is enough to cause inter-individual differences on cold tolerance.

Voluntary locomotion and stimulated behavior

The *A. sp.1 aff. fraterculus* had an overall low voluntary mobility at all tested temperatures, making difficult to determinate the effect of temperature on voluntary locomotion. Moreover, likewise on forced locomotion the null model was selected as one plausible model for voluntary walking, thus temperature, body size and sex might only have a small or none contribution for this behavior. This is probably associated with the fact that voluntary locomotion is more influenced by individuals' motivation than forced locomotion (Baškiera and Gvoždík, 2019). The effect of temperature on rates of voluntary activity may also vary with behavioral trait and context. Previous studies have found increased voluntary running speed of ants at higher temperatures (Lighton and Feened, 1989; Andrew *et al.,* 2013), while the ants' activity within the nest showed a modified Gaussian pattern as a function of temperature (Andrew *et al.,* 2013). We found that both males and females of *A. sp.1 aff. fraterculus* were more leaning to walk at intermediate temperatures (from 18 to 23°C) with a lower voluntary locomotion at 33°C, a pattern of thermal sensitivity similar to that found for forced locomotion. Additional studies incorporating repeated measurements of voluntary locomotion of *A. sp.1 aff. fraterculus* at different temperatures would be necessary to provide insights into the interference of individuals' motivation in thermal sensitivity curves of voluntary locomotion.

We also found that flies, especially the smaller ones, were more leaning to fly at temperatures within 18 to 33°C. Moreover, flies changed their body position and displayed cleaning behaviors more frequently at higher temperatures. Previous studies reported that *D. melanogaster* were only capable of lifting off and airborne for 1s at 15°C or higher environmental temperatures (Lehmann, 1999). It is important to consider that insects in general require a minimum body temperature in order to generate muscle mechanical power to flight, and that varies according to taxa (Dickinson and Ducley, 2009). Despite the absence of statistical effect of temperature on flying responses to stimulus, there was no report of flying responses to stimulus at 13°C. These results suggest that individuals of *A. sp.1 aff. frateculus* used in the present study cannot fly at 13°C. Additional studies testing thermal sensitivity of voluntary locomotion of individuals reared at different temperatures might be interesting to access the existence of plasticity in this trait.

Thermal sensitivity of aerobic metabolism

The aerobic metabolism was influenced by temperature; however, unlike locomotor performance that showed a clear U-inverted quadratic shape, there was an increase in CS activity with temperature. The activity of CS continued to increase with temperature up to 43°C, which was close to CT_{max} in males and higher than the CT_{max} in females of *A. sp.1 aff. fraterculus*. In general, the decrease phase of *TPC* is attributed to denaturation of proteins or disruption of protein interaction (Hochachka and Somero, 2002; Rezende and Bozinovic, 2019; Schutle *et al.*, 2011). However, our results showed that the decrease of locomotor performance at temperatures higher than 28.8±3.4^{o}C ^{o}C cannot be sole explained by decreasing activity of CS in these flies. According to metabolic control analysis (MCA) theory (Kacser, 1973), the control of metabolism emerges from small changes on metabolic pathways steps, each one mediated by an enzyme with different impact on flux control. Therefore, we cannot rule out the possibility that the emerging flux on the aerobic metabolism pathway might be different from the pattern found for isolated CS activity and determines, in some degree, thermal sensitivity of locomotor performance of *A. sp.1 aff. fraterculus*.

Under extreme thermal conditions, organisms are potentially subjected to cell-level damage. The exposures to low and high temperatures alter macromolecules functions, enzyme-binding affinity, and membrane fluidity, causing cell injury (see Denlinger and Yocum, 1998; see, Toxopeus and Sinclair, 2018). However, it is important to consider that thermal tolerance of organisms may be determined by higher levels of organization, involving processes associated with interactions between cells in a tissue and between systems (see Neven, 2000). Insect walking process does not rely only on energetic biochemical processes, but actually encompasses coordinative processes, leg movement generation, and systemic response to sensory feedback (Delcomyn, 2004). Recently, a selection event on CT_{min} of green anole lizards has been detected in response to extreme cold snap in southeastern United States (Campbell-Staton *et al.,* 2017). Interestingly, cold snap survivors showed up regulation of expression of proteins underlying synaptic function (Campbell-Staton *et al.,* 2017). Therefore, it is likely that thermal sensitivity of locomotor performance of *A. sp.1 aff. fraterculus* is dependent on the interaction of multiple processes in different organization levels, and the activity of nervous system might be particularly affected by temperature.

Our results showed that females had higher CS activity than males. Females of *A. fraterculus* are typically larger than males (Salles, 1995), and their higher aerobic activity might be particularly relevant due to their energetic demands for oviposition . Previous studies showed evidences that metabolic rates during locomotor activities in males of *Phoracantha spp.* might be associated with their necessity to travel long distances searching for possible mates (Rogowitz and Chappell, 2000). During ovipositon behavior, females of *Anastrepha* fruit flies need to find an appropriate host fruit, evaluate their nutritional fruit quality before lay their eggs and deposit host-marking pheromones on fruit surface after oviposition signalizing the presence of eggs for other visiting females (Aluja, 1999). Therefore, *A.sp.1 aff. fraterculus* female's higher aerobic activity might reflect a higher potential to activate and use their aerobic pathways to travel long distances and perform elaborate behaviors during oviposition. Although we did not test flight endurance, our results show possible relationships between behavioral traits associated with oviposition and flies aerobic metabolism.

Is jack-of-all-temperatures a master of none?

Our results showed only a non-significant correlation between maximum speed and breadth of locomotor performance in *A. sp.1 aff. fraterculus*. Thus, we did not corroborate the hypothesis '*jack-of-all-temperatures is a master of none*' for locomotor performance of these flies. Although this hypothesis has been previously corroborated for locomotor performance in a parasitic hymenopteran, with a negative genetic correlation between maximum speed and breadth of locomotor performance in males (Gilchrist, 1996). Other studies have also failed to find a trade-off to support this hypothesis in several traits of different organisms, including multiplication rates of unicellular organisms (Huey and Hertz, 1984), locomotor performance (Huey and Hertz, 1984) and fitness measurements of freshwater crustacean *Daphinia pulicaria* (Palaima and Spitze, 2004).

Huey and Hertz (1984) suggested that an absence of a trade-off could be explained by three plausible reasons. The first assumes that differences in health, condition and motivation have high influence on individuals' phenotype and can end up masking the trade-off. The second possibility implies that the principle of allocation involves trade-offs between multiple dimensions and cannot be detected in a two parameters analysis. The third possibility is that the trade-off is non-existent at

least for those individuals and parameters studied (Huey and Hertz, 1984). Even though all flies from our study were reared under the same environmental conditions (temperature, water and food *ad libitum*) we cannot discard the existence of motivation and condition variety among individual flies. It is also possible that other traits characterized by higher energetic costs, such as flight performance (Suarez, 2000), might show stronger evidence of trade-off between thermal optimum and thermal breath. Considering that understanding the evolutionary association between thermal optimum and thermal breath seems to be essential to estimate organisms' capacity to cope and adapt to a changing thermal environment (Hillaert *et al.,* 2015), we strongly encourage further studies investigating the heritability of these parameters and its responses to artificial selection to test the *'jack-of-all-temperatures is a master of none'* hypothesis.

In conclusion, males and females of the South-American fruit fly *A. sp.1 aff. fraterculus* showed a left-skewed inverted U-shaped thermal sensitivity curve of forced locomotor performance, with T_o ~ 28.7^{o}C, CT_{min} ~ 3.9 ^{o}C and CT_{max} ~ 43.1 ^{o}C. These flies are generally characterized by low frequency of voluntary locomotion within a large range of temperatures, but they are more leaning to walk at intermediate temperatures (18 to 23°C) than at higher temperatures (33°C). While gender affects heat tolerance (males show higher CT_{max} than females), body size affects cold tolerance (larger individuals show lower CT_{min}). The *in vitro* whole body maximum activity of CS showed an increase with temperature up to 43°C, which was closer to the CT_{max} in males and higher than the CT_{max} in females of *A. sp.1 aff. fraterculus*. These last results suggest that thermal sensitivity of total mitochondrial aerobic capacity is not an important isolated determinant of thermal sensitivity of locomotor performance in *A. sp.1 aff. fraterculus*. Finally, our results showed that V_{max} and T_{br} are not correlated, failing to corroborate the hypothesis *'jack-of-all-temperatures is a master of none'* for *A. sp.1 aff. fraterculus* locomotor performance.

5. Acknowledgments

6. References

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7. Appendices

Table A.1. Descriptive statistics of *Anastrepha sp.1 aff. fraterculus* body mass (mg), thermal limits (˚C), flies' mean walking speed (cm.s-1) and thermal performance curve parameters (T_o, V_{max}, B_{80} and T_{br}) for different sexes.

Sex	N	Variable	Mean	±	SD	Minimum	Maximum
Females	31	Body mass	1.54	±	0.17	1.19	1.96
		CT_{min}	3.85	±	0.87	2.59	6.40
		LP13	0.52	±	0.15	0.19	0.89
		LP18	0.87	±	0.27	0.28	1.52
		LP23	1.05	±	0.25	0.58	1.65
		LP28	1.38	±	0.36	0.18	2.10
		LP33	1.12	±	0.34	0.36	1.97
		CT_{max}	43.01	±	0.58	42.01	44.04
		T_o	28.76	±	3.35	20.50	37.50
		V_{max}	1.52	±	0.37	0.65	2.26
		B_{80}	10.93	±	3.82	2.04	17.24
		T_{br}	39.15	±	1.25	35.61	41.16
Males	27	Body mass	1.38	±	0.17	1.10	1.79
		CT_{min}	4.08	±	0.71	2.69	5.41
		LP13	0.50	±	0.18	0.20	1.01
		LP18	0.78	±	0.23	0.28	1.24
		LP23	0.92	±	0.30	0.41	1.49
		LP28	1.30	±	0.35	0.82	2.27
		LP33	1.01	±	0.27	0.41	1.53
		CT_{max}	43.30	±	0.51	41.98	44.49
		T_o	28.70	±	3.05	22.50	35.50
		V_{max}	1.39	±	0.53	0.82	3.46
		B_{80}	10.86	±	3.90	2.26	17.14
		T_{br}	39.22	±	0.78	37.93	40.89

CT_{min}: minimum critical temperature (˚C); **LP13**: walking speed at 13˚C (cm.s^{-1}); **LP18:** walking speed at 18˚C (cm.s^{-1}) ; **LP23:** walking speed at 23˚C(cm.s^{-1}); **LP28**: walking speed at 28˚C (cm.s^{-1}); **LP33**: walking speed at 33˚C (cm.s^{-1}); CT_{max}: maximum critical temperature (˚C);T_o: Optimum temperature(˚C); V_{max}: maximum speed (cm.s$^{-1)}$; B_{80}: temperature breath in witch 80% of maximum speed is achieved (˚C); T_{br}: Thermal performance breath (˚C); **F**: females; **M**: males.

Table A.2. Descriptive statistics of the scored behaviors of *Anastrepha sp.1 aff. fraterculus* (20 males and 20 females) at five different temperature (˚C)

Behavior	Sex	Temperature	Mean	±	SD	Minimum	Maximum
Walk	F	13	0.025	±	0.03	0.00	0.11
		18	0.024	±	0.05	0.00	0.22
		23	0.037	±	0.08	0.00	0.28
		28	0.052	±	0.13	0.00	0.59
		33	0.087	±	0.18	0.00	0.67
	M	13	0.027	±	0.06	0.00	0.28
		18	0.076	±	0.13	0.00	0.47
		23	0.021	±	0.04	0.00	0.17
		28	0.041	±	0.06	0.00	0.18
		33	0.040	±	0.11	0.00	0.41
Fly	F	13	0.000	±	0.00	0.00	0.00
		18	0.000	±	0.00	0.00	0.00
		23	0.001	±	0.00	0.00	0.01
		28	0.003	±	0.01	0.00	0.05
		33	0.000	±	0.00	0.00	0.01
	M	13	0.000	±	0.00	0.00	0.00
		18	0.002	±	0.01	0.00	0.02
		23	0.001	±	0.00	0.00	0.01
		28	0.001	±	0.00	0.00	0.01
		33	0.004	±	0.02	0.00	0.08
Stationary	F	13	0.922	±	0.05	0.82	0.98
		18	0.925	±	0.07	0.76	0.99
		23	0.919	±	0.09	0.66	0.99
		28	0.897	±	0.16	0.27	0.99
		33	0.854	±	0.20	0.23	0.98
	M	13	0.919	±	0.08	0.66	0.99
		18	0.881	±	0.13	0.46	0.98
		23	0.918	±	0.09	0.58	0.99
		28	0.903	±	0.08	0.73	0.99
		33	0.867	±	0.16	0.40	0.99
Body adjustment	F	13	4.950	±	4.44	0.00	16.00
		18	8.000	±	8.34	0.00	33.00
		23	6.300	±	6.01	0.00	20.00
		28	7.550	±	4.64	1.00	18.00
		33	7.950	±	4.25	4.00	17.00
	M	13	5.200	±	5.93	0.00	23.00
		18	8.050	±	9.12	0.00	31.00
		23	7.650	±	7.30	0.00	30.00
		28	12.850	±	8.84	1.00	36.00

			Mean	±	SD	Minimum	Maximum
		33	7.600	±	7.28	0.00	28.00
Cleaning	F	13	4.250	±	4.90	0.00	16.00
		18	5.700	±	5.49	0.00	19.00
		23	10.300	±	10.71	0.00	37.00
		28	12.950	±	8.99	0.00	31.00
		33	17.200	±	11.13	0.00	46.00
	M	13	5.250	±	4.06	0.00	14.00
		18	3.150	±	4.40	0.00	15.00
		23	6.300	±	7.04	0.00	23.00
		28	6.800	±	5.45	0.00	16.00
		33	10.550	±	11.72	0.00	39.00
Flying response	F	13	0.000	±	0.00	0.00	0.00
		18	0.005	±	0.02	0.00	0.10
		23	0.000	±	0.00	0.00	0.00
		28	0.015	±	0.05	0.00	0.20
		33	0.015	±	0.04	0.00	0.10
	M	13	0.000	±	0.00	0.00	0.00
		18	0.020	±	0.05	0.00	0.20
		23	0.015	±	0.04	0.00	0.10
		28	0.025	±	0.08	0.00	0.30
		33	0.005	±	0.02	0.00	0.10

Walk: proportion on time flies spent walking; **Fly**: proportion of time flies spent flying; **Stationary**: proportion of time flies spent stationary; **Body adjustment**: number of times flies changed their body position and **Cleaning**: number of times flies cleaned their wings and head; **F**: females; **M**: males

Table A.3. Descriptive statistic of citrate synthase activity ($umol.min.^{-1}g$ $tissue^{-1}$) of *Anastrepha sp.1 aff. fraterculus* at different temperatures.

Sex	N	Temperature	Mean	±	SD	Minimum	Maximum
F	9	13	33.27	±	10.50	19.46	50.19
		23	51.95	±	14.87	31.42	79.14
		33	79.74	±	21.47	49.12	110.47
		43	106.86	±	31.47	68.44	163.57
M	8	13	26.68	±	5.33	16.86	33.66
		23	47.26	±	11.71	31.31	67.37
		33	68.56	±	15.53	45.15	85.99
		43	93.19	±	22.90	59.84	137.26

F: females; **M**: males

Considerações Finais

Através do nosso estudo vimos que a curva de desempenho térmico da locomoção forçada de machos e fêmeas de *A. sp.1 aff. fraterculus* pode ser descrita por uma curva em forma de U-invertido deslocada para a esquerda, e determinamos seus principais parâmetros como a $T_o \sim 28.7\,^{\circ}C$, $CT_{min} \sim 3.9\,^{\circ}C$ e $CT_{max} \sim 43.1\,^{\circ}C$. As moscas movimentaram-se voluntariamente com menor frequência se comparado à locomoção forçada em todas as temperaturas testadas. Apesar disso, pudemos observar que as moscas apresentaram uma tendência maior de andarem voluntariamente nas temperaturas intermediárias 18 e $23\,^{\circ}C$ do que à $33\,^{\circ}C$. Em nosso estudo também observamos que as fêmeas foram mais tolerantes ao calor do que machos, enquanto indivíduos maiores foram mais tolerantes ao frio.

O teste *in vitro* da atividade máxima da citrato sintase mostrou um aumento da atividade enzimática em função da temperatura. Tendo em vista que a maior temperatura testada foi $43\,^{\circ}C$, a qual é próxima da CT_{max} de machos e superior ao CT_{max} de fêmeas, nossos resultados indicam que a capacidade aeróbica mitocondrial total não é o único determinante da sensibilidade térmica do desempenho locomotor desta espécie.

Por fim, nosso estudo mostrou que V_{max} e T_{br} não têm uma correlação significativa. Desta forma, refutamos a hipótese *"jack-of-all-temperatures is a master of none"* para o desempenho locomotor de *A. sp.1 aff. fraterculus*.