DIEGO DE SOUZA

MORPHOMETRY APPLIED TO COMMERCIAL *TRICHOGRAMMA* spp. MASS REARING

LAVRAS – MG

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Tese apresentada à Universidade Federal de Lavras, como parte das exigências do Programa de Pós-Graduação em Entomologia, área de concentração Entomologia, para obtenção do título de Doutor em ciências.

Prof. Dr. Lucas Del Bianco Faria

Orientador

Prof. Dr. Geraldo Andrade Carvalho

Coorientador

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DIEGO DE SOUZA

MORPHOMETRY APPLIED TO COMMERCIAL *TRICHOGRAMMA* spp. MASS REARING

MORFOMETRIA APLICADA À CRIAÇÃO MASSAL COMERCIAL DE *TRICHOGRAMMA* spp.

Tese apresentada à Universidade Federal de Lavras, como parte das exigências do Programa de Pós-Graduação em Entomologia, área de concentração Entomologia, para obtenção do título de Doutor em ciências.

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Dr. Lucas Del Bianco Faria (UFLA)

Dr. Bruno Henrique Sardinha de Souza (UFLA)

Dra. Rosangela Cristina Marucci (UFLA)

Dr. Wesley Augusto Conde Godoy (USP-ESALQ)

Dr. Gustavo Rodrigues Alves (Koppert)

Prof. Dr. Lucas Del Bianco Faria

Orientador

Prof. Dr. Geraldo Andrade Carvalho

Coorientador

LAVRAS – MG

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RESUMO

A qualidade de um inimigo natural é medida de diferentes formas, sendo o tamanho dos organismos um importante parâmetro. O tamanho do adulto muitas vezes é determinado pelo comprimento da tíbia posterior, entretanto, a escolha das características a serem mensuradas deve ser feita de forma criteriosa, visto que utilizar apenas uma para estimar o tamanho dos organismos pode levar a interpretações incertas. Os fatores que influenciam diretamente o tamanho dos parasitoides oófagos são o número de indivíduos que desenvolvem em um único hospedeiro e o tamanho do mesmo. Desta forma, a relação entre o tamanho do ovo do hospedeiro e o do parasitoide também é um parâmetro importante a ser determinado para avaliação da qualidade de um parasitoide. Entender quais fatores podem influenciar o tamanho dos ovos dos hospedeiros é importante para a produção de recursos utilizados na criação de parasitoides. Neste trabalho foi avaliado a distribuição de tamanho dos parasitoides *Trichogramma pretiosum* e *Trichogramma galloi* com o intuito de determinar qual parte do corpo melhor representa a variação no seu tamanho. Para isso, espécimes foram montados em lâminas para microscopia, fotografados e as fotos foram utilizadas para medir estruturas morfológicas. Insetos grandes e pequenos foram separados com base na variação de tamanho de suas características morfológicas e as melhores foram utilizadas para representar a variação de tamanho dos parasitoides. Ovos do hospedeiro *Ephestia kuehniella* foram fotografados para estimar o seu tamanho e relacionar com os maiores parasitoides emergidos. Também, o efeito das temperaturas 20ºC, 22.5ºC e 25ºC no tamanho de ovos do hospedeiro alternativo foi avaliado. Com base em análises de componentes principais e análise discriminante, conclui-se que o comprimento da asa e do ovipositor são as melhores partes para representar a variação de tamanho dos parasitoides *T. pretiosum* e *T. galloi*. Os parasitoides grandes emergem dos maiores ovos do hospedeiro; entretanto, de um ovo grande não é garantida a emergência de um espécime grande. As temperaturas experimentadas pelo hospedeiro *E. kuehniella* durante seu desenvolvimento imaturo e na fase adulta afetam o tamanho de seus ovos, sendo maiores na temperatura de 20ºC.

Palavras-chave: Controle biológico. Parasitoides. Hospedeiros. Ovos. Controle de qualidade.

ABSTRACT

The quality of a natural enemy is measured in different ways, being the size of organisms an important parameter. The adult's body size is often determined by the length of the posterior tibia, however, the choice of characteristics to be measured must be done carefully, since using only one to estimate the size of organisms can lead to misunderstandings. The factors that directly influence the size of oophagous parasitoids are the number of individuals that develop in a single host and the host size. Thus, the relationship between the host's egg size and the parasitoid's size is also an important parameter to be determined for evaluating the quality of a parasitoid. Understanding which factors can influence the host's egg size is important to produce resources used in the mass rearing of parasitoids. In this study, the size distribution of the parasitoids *Trichogramma pretiosum* and *Trichogramma galloi* was evaluated in order to determine which body part best represents the variation in its size. Therefore, specimens were mounted on slides for microscopy, photographed and the photos were used to measure morphological structures. Large and small specimens were separated based on the size span of their morphological characteristics and the best ones were used to represent the size span of the parasitoids. Eggs of the host *Ephestia kuehniella* were photographed to estimate their size and relate to the largest parasitoids emerged. Also, the effect of temperatures 20ºC, 22.5ºC and 25ºC on the *E. kuehniella* egg size was evaluated. Based on principal component analysis and discriminant analysis, we concluded that the wing and ovipositor lengths best represent the variation in size of the parasitoids *T. pretiosum* and *T. galloi*. Large parasitoids emerge from the host's largest eggs; however, from a large egg the emergence of a large specimen is not guaranteed. The temperatures experienced by the host *E. kuehniella* during its immature development and in the adult phase affect the size of its eggs, being bigger at a temperature of 20ºC.

Keywords: Biological control. Parasitoids. Hosts. Eggs. Quality control.

SUMÁRIO

PRIMEIRA PARTE

INTRODUÇÃO GERAL

O uso de insumos biológicos cresce em todo mundo, e no Brasil, que é o líder mundial na utilização de controle biológico em condições de campo, as perspectivas são de crescimento acelerado. A qualidade dos organismos biológicos produzidos em laboratório é de extrema importância para o seu sucesso e ela pode ser relacionada ao tamanho dos organismos produzidos. O tamanho dos parasitoides de ovos produzidos em laboratório muitas vezes é determinado pelo comprimento da tíbia posterior, entretanto, utilizar apenas uma característica para estimar o tamanho dos organismos pode levar a diferentes interpretações e, por isso, é importante determinar quais características melhor representam a variação de tamanho desses organismos.

Alguns fatores podem influenciar o tamanho dos parasitoides, e os mais importantes são o número de indivíduos que desenvolvem em um único hospedeiro e o tamanho do hospedeiro. Entender quais fatores podem influenciar o tamanho dos ovos dos hospedeiros é importante para a produção de recursos utilizados na criação de parasitoides. Desta forma, entender relação entre o tamanho do ovo do hospedeiro e o do parasitoide é importante para avaliar a qualidade de um parasitoide e otimizar a sua criação em grandes quantidades.

A espécie *Ephestia kuehniella* Zeller, 1879 (Lepidoptera: Pyralidae) é o hospedeiro alternativo mais utilizado nas criações de inimigos naturais e sua qualidade pode influenciar a qualidade das criações. De acordo com a hipótese de “depleção de recursos”, as fêmeas tendem a investir maiores recursos em suas primeiras progênies para aumentar o sucesso reprodutivo, com isso, utilizar os primeiros ovos colocados nas criações de parasitoides pode ser uma estratégia para melhorar as criações. Além disso, a regra “temperatura-tamanho” postula que baixa temperatura durante o período de oviposição induz as fêmeas a colocarem ovos maiores, os quais podem ter maior qualidade.

Esta tese busca informações que possam colaborar para o aprimoramento de técnicas de criação de inimigos naturais levando em consideração que parasitoides maiores são de maior qualidade, assim, a correta classificação e seleção em tamanho dos parasitoides de ovos pode gerar organismos mais eficientes para o controle biológico de pragas. Além disso, o fornecimento de recursos de maior qualidade para a criação de parasitoides pode levar a produção de inimigos naturais cada vez melhores, contribuindo para o controle biológico.

REFERENCIAL TEÓRICO

O interesse pelos insumos biológicos cresce em todo mundo. No Brasil, que é o líder mundial na utilização de controle biológico em condições de campo, o faturamento do setor chegou a R$ 1,74 bilhão em 2021; R$ 3,3 bilhões em 2022; com perspectivas de atingir R$ 8,08 bilhões em 2030, e cerca de US $18,5 bilhões em todo o mundo, representando um crescimento de 74% em quatro anos (Dall'Agnol 2022; Gottems 2022; Herrmann 2023). Em 2023, algumas empresas do setor de produção de bioinsumos irão captar até R$ 200 milhões para ampliarem suas estruturas, e para os próximos 3 anos cerca de 700 milhões podem ser investidos na América do Sul (Herrmann 2023). Vários fatores impulsionam o setor, destacando-se a inocuidade dos agentes biológicos ao ambiente e à saúde das pessoas; a isenção de resíduos nos alimentos comercializados; a aderência aos programas de baixa emissão de carbono, contribuindo para a filosofia ESG (Environmental, Social and Governance), e principalmente, a efetividade dos agentes biológicos (Vital 2021). Com isso, para que o controle biológico prospere, produzir insumos biológicos de qualidade é de extrema importância.

Em biologia existe o conceito de *fitness* que, de forma resumida, significa a capacidade de um espécime em produzir descendentes (Bolhuis e Giraldeau 2005). Esse conceito pode ser utilizado na área de controle biológico de pragas como a capacidade ou habilidade de um inimigo natural em localizar seu alimento e, no caso de parasitoides oófagos, a habilidade em localizar os ovos de seus hospedeiros em condições de campo e parasitá-los. Desta forma, quanto maior o *fitness* dos inimigos naturais, mais alta será sua qualidade e, consequentemente, maior será sua capacidade em controlar as pragas. Muitos pesquisadores ressaltam que a qualidade dos organismos biológicos produzidos em laboratório é de extrema importância para o sucesso de programas de controle biológico em campo. Desta forma, é importante selecionar os melhores, mais rápidos e mais confiáveis métodos de se avaliar a qualidade de um inimigo natural (Van Lenteren 2003).

O controle de qualidade é uma peça fundamental nos programas de criação massal, e tem como objetivos identificar problemas de produção, monitorar a qualidade das linhagens criadas e garantir a qualidade do produto final. A qualidade total de um inimigo natural pode ser definida como a capacidade deste em executar seu papel após a liberação em campo. Desta forma, o controle de qualidade busca checar se a qualidade total está sendo mantida, e para isso o controle de qualidade interrelaciona o controle de qualidade da produção, do processo e do produto, cada um com suas próprias particularidades. O controle de qualidade da produção garante que as operações de criação estão sendo realizadas e monitoradas de acordo com os

equipamentos, procedimentos e ambiente corretos. O controle de qualidade no processo busca ajustar os procedimentos de criação por meio da avaliação do produto inacabado. Por fim, o controle de qualidade do produto busca assegurar que os insetos criados possuem condições apropriadas para tratamento, manuseio e transferência para utilização (Van Lenteren 2003).

A qualidade de um inimigo natural produzido em laboratório é medida pela quantidade, que representa o número de insetos vivos que foram produzidos e serão liberados; razão sexual, que diz respeito à porcentagem mínima de fêmeas produzidas; fecundidade, que representa o número de descendentes produzidos durante determinado período de tempo, sendo desejado que este número seja alto; longevidade, que é o número de dias que o organismo adulto produzido vive e espera-se que seja suficiente para que o inimigo natural cumpra seu papel no controle biológico; predação, que significa o número de presas consumidas durante determinado período de tempo; tamanho do adulto, que na maioria das vezes é determinado pelo tamanho da tíbia posterior; atividade de voo, englobando voo de curta e longa duração; e por fim, desempenho em campo, que se resume na capacidade do inimigo natural produzido em laboratório de localizar, predar a sua presa ou alimentar-se de seu hospedeiro (Van Lenteren 2003).

Muitos parâmetros de qualidade estão associados ao tamanho dos inimigos naturais produzidos, que, como mencionado, também faz parte da avaliação da qualidade, visto que é um parâmetro que tem covariação positiva com a fisiologia e comportamento de inimigos naturais (Brown et al. 2004; Li e Zhang 2018). O tamanho da tíbia posterior esquerda é positivamente correlacionado com a longevidade e fecundidade do parasitoide *Anagyrus kamali* Moursi, 1948 (Himenoptera: Encyrtidae), demonstrando que insetos maiores ovipositam em média 5 ovos a mais que os pequenos. Durante a vida reprodutiva, as maiores fêmeas ovipositam cerca de 88 ovos a mais do que as menores, refletindo em uma taxa de parasitismo aproximadamente 15% maior para fêmeas grandes (Sagarra et al. 2001). A localização do hospedeiro no campo também está diretamente relacionada com o tamanho dos inimigos naturais (Clarke e McKenzie 1992; Kazmer e Luck 1995; West et al. 1996; Bennett e Hoffmann 1998; Fournier e Boivin 2000; Sagarra et al. 2001; Kölliker-Ott et al. 2003). Considerando a hipótese de que a habilidade em encontrar hospedeiros é dependente do tamanho do parasitoide, avaliar a diferença entre o tamanho das fêmeas liberadas no campo e o tamanho das fêmeas encontradas no momento de oviposição pode ser um indicativo da eficiência no controle biológico.

A distribuição de tamanho dos parasitoides *Trichogramma brassicae* Bezdenko, 1968 (Himenoptera: Trichogrammatidae) e *Trichogramma pretiosum* Riley, 1879 (Himenoptera: Trichogrammatidae) liberados em campo foi analisada em função do tamanho dos parasitoides que ovipositaram em seus hospedeiros. Das 2000 fêmeas recém emergidas liberadas de cada espécie, apenas 91 de *T. brassicae* e 72 de *T. pretiosum* foram observadas parasitando os ovos de *Helicoverpa armigera* Hubner, 1808 (Lepidoptera: Noctuidae) em campo. Verificaram que fêmeas com asas maiores apresentaram maior capacidade de localizar seus hospedeiros, e isso indica uma seleção natural para parasitoides de maior tamanho (Kölliker-Ott et al. 2003). A localização do hospedeiro está diretamente relacionada com a habilidade de voo dos insetos, com isso, pequenas alterações no tamanho e forma das asas podem ter um grande impacto na capacidade de seu voo (Kölliker-Ott et al. 2003). O sucesso de *T. pretiosum* na localização de hospedeiros em campo aumenta com o tamanho dos parasitoides pequenos; porém, se estabiliza quando os parasitoides são maiores, indicando a existência de um tamanho ótimo para sua melhor performance (Kazmer e Luck 1995). *Trichogramma carverae* Oatman e Pinto, 1987 (Himenoptera: Trichogrammatidae) também foi avaliado em campo e apresentou correlação positiva entre tamanho e sucesso na localização e parasitismo de hospedeiros (Bennett e Hoffmann 1998). As fêmeas naturais de *T. carverae* capturadas durante o parasitismo eram maiores do que as fêmeas liberadas, e mais importante, muitas fêmeas encontradas durante a oviposição foram classificadas como *outliers* quando comparadas à distribuição de tamanho da amostra de fêmeas liberadas. Estas fêmeas capturadas durante o parasitismo representavam cerca de 27% dos parasitoides capturados, enquanto representavam apenas 0,04% dos parasitoides liberados no campo (Bennett e Hoffmann 1998).

Embora trabalhos de literatura apresentam relação positiva entre tamanho e desempenho em campo, há casos que essa relação não é muito clara. O parasitoide *T. brassicae* foi avaliado em casa de vegetação e neste caso não foi observada diferença entre o tamanho das tíbias dos parasitoides liberados com aqueles que encontraram o hospedeiro *Helicoverpa punctigera* Wallengren, 1860 (Lepidoptera: Noctuidae). Entretanto, foi observado que insetos com tíbias de tamanho intermediário foram os que apresentaram o melhor desempenho em casa de vegetação, sendo que aqueles pequenos e grandes tiveram menor habilidade para encontrar seu hospedeiro (Hewa-Kapuge e Hoffmann 2001). Por outro lado, quando *T. carverae* foi avaliado em campo, parasitoides com tamanho intermediário apresentaram o menor desempenho e os com tíbias medindo acima de 0,14 mm e menores que 0,12 mm foram os mais eficientes na localização do hospedeiro *Epiphyas postvittana* Walker, 1863 (Lepidoptera: Tortricidae)

(Bennett e Hoffmann 1998). Quando foram avaliados parasitoides criados no hospedeiro *Sitotroga cerealella* Oliver, 1789 (Lepidoptera: Gelechiidae) e *Helicoverpa zea* Boddie, 1850 (Lepidoptera: Noctuidae), resultados contraditórios também foram verificados. A espécie *T. pretiosum* foi criada em ovos de *H. zea* com o objetivo de se obter parasitoides maiores e em ovos de *S. cerealella* para gerar parasitoides pequenos (Kazmer e Luck 1995). Neste estudo observou-se que os menores parasitoides tiveram maior capacidade em localizar e parasitar hospedeiros em campo. Desta forma, foi verificado que pode ocorrer a interação entre a espécie do parasitoide com a do hospedeiro, além de que as correlações entre tamanho e *fitness* podem ser diferentes quando se considera condições de laboratório ou de campo.

Medir os organismos é uma prática simples, porém, escolher a parte a ser mensurada deve ser feita de forma criteriosa, visto que apenas uma característica para estimar o tamanho dos organismos pode levar a equívocos. A espécie *T. pretiosum* foi categorizada em dez classes baseadas no tamanho de suas tíbias e asas. Em seguida, os espécimes das classes 7, 8 e 9 foram agrupados para criar um grupo de parasitoides grandes, e as classes 2, 3 e 4 para criar um grupo com parasitoides pequenos. Não foi possível obter grupos com parasitoides grandes e pequenos baseado somente em uma característica. Para se obter dois grupos distintos foi necessário a utilização de mais de uma característica, pois o mesmo inseto que é considerado como grande quando se utiliza a tíbia pode ser classificado como pequeno quando a mensuração é feita somente na asa. Isso pode levar a equívocos quando se pretende estudar o efeito do tamanho desses organismos com sua performance em condições de campo (Souza e Faria 2022).

Alguns fatores influenciam no tamanho dos parasitoides. A espécie do hospedeiro alternativo e sua qualidade podem influenciar o tamanho, sendo que espécies que produzem ovos maiores e com maior valor nutricional tendem a produzir parasitoides maiores (Vos e Vet 2004). A idade do hospedeiro alternativo no momento do parasitismo também influencia no seu tamanho visto que ovos com idade avançada podem ter seus recursos consumidos pelo embrião do hospedeiro alternativo, restando poucas reservas para o parasitoide (Godin e Boivin 2000). O tempo de desenvolvimento do parasitoide é outro fator que influenciam no tamanho, e esta relação é explicada principalmente pela regra Temperatura-Tamanho, onde organismos que se desenvolvem em baixas temperaturas têm seu tempo de desenvolvimento aumentado fazendo com que os organismos fiquem maiores (Angilletta et al. 2004). Mas podemos dizer que são dois os principais fatores que influenciam o tamanho dos parasitoides oófagos, o número de indivíduos dentro do hospedeiro e o tamanho do hospedeiro. As fêmeas que emergem de hospedeiros maiores são maiores, mais fecundas, vivem mais e têm maior sucesso na

localização de hospedeiros em campo do que aquelas que surgem de hospedeiros menores (Bai et al. 1992; Kazmer e Luck 1995). Além disso, sabe-se que diferentes hospedeiros são caracterizados por distintos conteúdos nutricionais e tamanhos (Cascone et al. 2015), sendo que uma pequena variação no tamanho do hospedeiro, afeta o tamanho do parasitoide dele emergido, e isso pode ter impacto positivo na sua longevidade (Alcalá et al. 2021). Desta forma, a relação entre o tamanho do ovo do hospedeiro e o tamanho do parasitoide emergido pode ser um parâmetro importante a ser avaliado, além de se buscar entender quais fatores podem influenciar o tamanho dos ovos dos hospedeiros.

De acordo com a hipótese de "depleção de recursos", os maiores ovos são colocados primeiro devido a um esgotamento progressivo das reservas alimentares maternas durante a produção de ovos, o que resulta em uma diminuição do esforço reprodutivo com a idade da fêmea, ocorrendo redução na fecundidade e no tamanho do ovo (Campbell 1962; Wellington 1965; Boggs 1986; Fox e Czesak 2000; Fischer et al. 2003a). As fêmeas tendem a investir maiores recursos em suas primeiras progênies para aumentar o sucesso reprodutivo, enfatizando a hipótese de "depleção de recursos" (Karlsson e Wiklund 1984; Karlsson 1987).

Na criação massal de *Ephestia kuehniella* Zeller, 1879 (Lepidoptera: Pyralidae), a postura ocorre ao longo de quatro a sete dias, mas a maioria dos ovos é colocada nos primeiros quatro dias (Parra et al. 2014). As fêmeas de *Chilo partellus* Charles Swinhoe, 1885 (Lepidoptera: Pyralidae) colocam ovos maiores no primeiro dia de vida, sendo que aquelas maiores ovipositam ovos maiores (Berger 1989). O número de ovos diminui com a idade da fêmea; entretanto, há pouca informação sobre o tamanho do ovo, sabendo-se que seu peso permaneceu constante em alguns casos (Berger 1989), e diminuiu na maioria dos estudos (Harvey 1977; Jones et al. 1982; Karlsson e Wiklund 1984; Kimura e Tsubaki 1985; Boggs 1986; Wickman e Karlsson 1987; Ruohomäki et al. 1993; Oberhauser 1997). Espera-se que o tamanho do ovo varie em função da idade das fêmeas em fase reprodutiva (Wiklund e Persson 1983).

Fatores ambientais podem afetar o tamanho do ovo (Harvey 1983; Fischer et al. 2004; Steigenga et al. 2005; Amiri et al. 2020). Por exemplo, rainhas de *Apis mellifera* Linnaeus, 1758 (Hymenoptera: Apidae) tendem a produzir ovos maiores em colônias grandes e com restrição de pólen, supondo que condições menos ideais induzam rainhas a investir em ovos maiores para aumentar a sobrevivência de sua progênie (Harvey 1977; Vijendravarma et al. 2010; Amiri et al. 2020). A temperatura experimentada durante as fases imatura e adulta pode afetar o tamanho do ovo. Em temperaturas mais baixas geralmente os ovos são maiores (Campbell 1962; Harvey

1983; Fox e Czesak 2000; Steigenga et al. 2005). De acordo com Harvey (1983) e Fischer et al. (2004) o efeito direto da temperatura no tamanho do ovo ocorre nos últimos instares, visto que o desenvolvimento dos ovaríolos ocorre nesse período. No entanto, foi descoberto que adultos expostos às variações desse fator abiótico podem colocar ovos de diferentes tamanhos (Fischer et al. 2003b; Steigenga et al. 2005). Observou-se que a baixa temperatura durante o período de oviposição induz as fêmeas a colocarem ovos maiores (Fischer et al. 2003b; Fischer et al. 2004). Esses estudos foram feitos com insetos que têm maior longevidade, os quais podem ajustar sua fisiologia em resposta à temperatura para produzir ovos grandes (Fischer et al. 2003a). Como *E. kuehniella* tem menor longevidade, o efeito da temperatura no tamanho do ovo ocorre no estágio final da fase imatura, sendo que o adulto recém formado apresenta características que permitem resposta plástica quanto ao tamanho em função da variação da temperatura (Fischer et al. 2003b). Portanto, a temperatura e o dia que o ovo é colocado são dois fatores críticos que afetam o tamanho do ovo e podem ser manejados durante a criação massal de *E. kuehniella*, a fim de obter melhores resultados quanto ao tamanho dos ovos produzidos.

Esta tese trabalha com a premissa de que insetos maiores tem maior qualidade e, considerando que um dos principais fatores que influenciam no tamanho dos parasitoides de ovos é o tamanho do ovo do hospedeiro, aumentar o tamanho dos ovos pode ser uma alternativa para produzir parasitoides de maior tamanho. Com isso, o primeiro capítulo desta tese tem como objetivo determinar qual parte do corpo melhor representa a variação de tamanho dos parasitoides *T. pretiosum* e *Trichogramma galloi* Zucchi, 1988 (Hymenoptera: Trichogrammatidae); e o segundo capítulo desta tese tem como objetivos: avaliar a relação entre o tamanho do parasitoide adulto com o tamanho do ovo do hospedeiro alternativo, testando a hipótese de que parasitoides grandes emergem dos maiores ovos, e avaliar a possibilidade de manipulação do tamanho do ovo do hospedeiro alternativo *E. kuehniella* em função das variações de temperatura em sala de criação, testando a hipótese de que ovos maiores são colocados em temperaturas menores.

CONSIDERAÇÕES GERAIS

Cada vez mais aumenta a preocupação com os impactos negativos causados pelos agrotóxicos ao ambiente e ao homem, o que vem comprometendo a sustentabilidade do setor agrícola brasileiro. Em função disso, programas de controle biológico vem sendo cada vez mais aceitos por produtores rurais como uma medida viável e sustentável de controle de artrópodes pragas nos diferentes agroecossistemas. Desta forma, estudos que busquem informações que

possam colaborar para o aprimoramento de técnicas de criação de inimigos naturais devem ser incentivados, visto que o controle biológico atende aos preceitos de agricultura regenerativa.

O tamanho dos inimigos naturais produzidos comercialmente é importante para determinar a sua qualidade. Parasitoides grandes são considerados como mais eficientes no controle de pragas; com isso, a correta avaliação do seu tamanho é de grande importância para a garantia de qualidade de uma criação. No presente trabalho verificou-se que a avaliação do tamanho dos parasitoides *T. pretiosum* e *T. galloi* deve ser feita de forma representativa e assertiva para evitar encontrar informações incertas. Ao se avaliar o tamanho de um espécime é importante conhecer o seu comportamento de dispersão em campo e identificar as características morfológicas mais importantes responsáveis pela otimização de sua performance em programas de controle biológico, além de identificar os fatores que afetam o seu tamanho.

Em parasitoides oófagos, o tamanho dos ovos de seu hospedeiro é um fator que influencia o seu tamanho. No presente estudo, observou-se que dos ovos maiores do hospedeiro alternativo *E. kuehniella* emergiram espécimes maiores de *T. pretiosum* e de *T. galloi*. Embora essa relação possa variar e nem sempre ovos maiores produzirão parasitoides grandes, utilizar ovos maiores na criação de parasitoides pode ser uma forma de melhorar a sua qualidade e, consequentemente, otimizar sua eficiência em programas de controle biológico de lepidópteros pragas.

O custo de produção envolvido na criação de hospedeiros alternativos é um dos fatores limitantes para a criação de alguns grupos de inimigos naturais. Diferentes dietas são testadas com a finalidade de reduzir o custo de produção e melhorar os parâmetros biológicos associados aos hospedeiros alternativos. Os ovos do lepidóptero *E. kuehniella* são utilizados em várias partes do mundo como recursos alimentares, visando a criação de diferentes espécies de parasitoides e predadores, e desta forma, é importante a otimização de produção desse recurso. Nós demonstramos que a temperatura no ambiente de 20ºC em que as pupas do hospedeiro alternativo *E. kuehniella* foi criado proporcionou a produção de ovos maiores.

As informações obtidas no presente trabalho poderão ser úteis para o aprimoramento de criações comerciais dos parasitoides *T. pretiosum* e *T. galloi* e de seu hospedeiro *E. kuehniella*. Procedimentos que busquem produzir ovos maiores do hospedeiro permitirão a produção de maiores parasitoides, os quais serão mais eficientes na localização e parasitismo de seus hospedeiros em condições de campo.

REFERÊNCIAS

Acevedo-Alcalá, A., Rodríguez-Leyva, E. and Lomeli-Flores, J.R., 2021. Impact of Host Size and Honey Availability on *Trichogramma atopovirilia* (Hymenoptera: Trichogrammatidae) Longevity. Journal of Entomological Science, 56(3), p.458-461.

Amiri, E., Le, K., Melendez, C.V., Strand, M.K., Tarpy, D.R. and Rueppell, O., 2020. Egg-size plasticity in *Apis mellifera*: honey bee queens alter egg size in response to both genetic and environmental factors. Journal of Evolutionary Biology, 33(4), p.534-543.

Angilletta Jr, M.J., Steury, T.D. and Sears, M.W., 2004. Temperature, growth rate, and body size in ectotherms: fitting pieces of a life-history puzzle. Integrative and comparative biology, 44(6), p.498-509.

Bai, B., Luck, R.F., Forster, L., Stephens, B. and Janssen, J.M., 1992. The effect of host size on quality attributes of the egg parasitoid, *Trichogramma pretiosum*. Entomologia Experimentalis et Applicata, 64(1), p.37-48.

Berger, A., 1989. Egg weight, batch size and fecundity of the spotted stalk borer, *Chilo partellus* in relation to weight of females and time of oviposition. Entomologia Experimentalis et Applicata, 50(3), p.199-207.

Bennett, D.M. and Hoffmann, A.A., 1998. Effects of size and fluctuating asymmetry on field fitness of the parasitoid *Trichogramma carverae* (Hymenoptera: Trichogrammatidae). Journal of Animal Ecology, p.580-591.

BOGGS, C.L., 1986. Reproductive strategies of female butterflies: variation in and constraints on fecundity. Ecological Entomology, 11(1), p.7-15.

Bolhuis, J.J. and Giraldeau, L.A.E., 2005. The behavior of animals: Mechanisms, function, and evolution. Blackwell Publishing.

Brown, J.H., Gillooly, J.F., Allen, A.P., Savage, V.M. and West, G.B., 2004. Toward a metabolic theory of ecology. Ecology, 85(7), p.1771-1789.

Campbell, I.M., 1962. Reproductive capacity in the genus *Choristoneura* Led. (Lepidoptera: Tortricidae). I. Quantitative inheritance and genes as controllers of rates. Canadian Journal of Genetics and Cytology, 4(3), p.272-288.

Clarke, G.M. and McKenzie, L.J., 1992. Fluctuating asymmetry as a quality control indicator for insect mass rearing processes. Journal of Economic Entomology, 85(6), p.2045-2050.

Cascone, P., Carpenito, S., Slotsbo, S., Iodice, L., Sørensen, J.G., Holmstrup, M. and Guerrieri, E., 2015. Improving the efficiency of *Trichogramma achaeae* to control *Tuta absoluta*. BioControl, 60(6), p.761-771.

Dall'Agnol, A. Mercado de insumos biológicos em crescimento. Canal Rural, 2022. Disponível em: https://blogs.canalrural.com.br/embrapasoja/2022/05/30/mercado-de-insumos-biologicos-em-crescimento/. Acesso em: 26/01/2023.

Fischer, K., Brakefield, P.M. and Zwaan, B.J., 2003a. Plasticity in butterfly egg size: why larger offspring at lower temperatures? Ecology, 84(12), p.3138-3147.

Fischer, K., Eenhoorn, E., Bot, A.N., Brakefield, P.M. and Zwaan, B.J., 2003b. Cooler butterflies lay larger eggs: developmental plasticity versus acclimation. Proceedings of the Royal Society of London. Series B: Biological Sciences, 270(1528), p.2051-2056.

Fischer, K., Bot, A.N.M., Zwaan, B.J. and Brakefield, P.M., 2004. Genetic and environmental sources of egg size variation in the butterfly *Bicyclus anynana*. Heredity, 92(3), p.163-169.

Fournier, F. and Boivin, G., 2000. Comparative dispersal of *Trichogramma evanescens* and *Trichogramma pretiosum* (Hymenoptera: Trichogrammatidae) in relation to environmental conditions. Environmental Entomology, 29(1), p.55-63.

Fox, C.W. and Czesak, M.E., 2000. Evolutionary ecology of progeny size in arthropods. Annual Review of Entomology, 45(1), p.341-369.

Godin, C. and Boivin, G., 2000. Effects of host age on parasitism and progeny allocation in Trichogrammatidae. Entomologia Experimentalis et Applicata, 97(2), pp.149-160.

Gottems, L. Mercado de biológicos deve disparar 74% em 4 anos. AGRO LINK, 2022. Disponível em: https://www.agrolink.com.br/noticias/mercado-de-biologicos-deve-disparar-74--em-4-anos_461025.html?utm_source=agrolink-clipping&utm_medium=email&utm_campaign=clipping_edicao_7082&utm_content=noticia&ib=y. Acesso em: 26/01/2023.

Harvey, G.T., 1977. Mean weight and rearing performance of successive egg clusters of eastern spruce budworm (Lepidoptera: Tortricidae). The Canadian Entomologist, 109(4), p.487-496.

Harvey, G.T., 1983. Environmental and genetic effects on mean egg weight in spruce budworm (Lepidoptera: Tortricidae). The Canadian Entomologist, 115(9), p.1109-1117.

Herrmann, G. Koppert usa Rabobank para captar FIDC de R$ 200 milhões. Forbes Agro, 2023. Disponível em: https://forbes.com.br/forbesagro/2023/01/koppert-usa-rabobank-para-captar-fidc-de-r-200-milhoes/. Acesso em: 26/01/2023.

Hewa-Kapuge, S. and Hoffmann, A.A., 2001. Composite asymmetry as an indicator of quality in the beneficial wasp *Trichogramma* nr. *brassicae* (Hymenoptera: Trichogrammatidae). Journal of Economic Entomology, 94(4), p.826-830.

Jones, R.E., Hart, J.R. and Bull, G.D., 1982. Temperature, size and egg production in the cabbage butterfly, *Pieris rapae* L. Australian Journal of Zoology, 30(2), p.223-231.

Karlsson, B. and Wiklund, C., 1984. Egg weight variation and lack of correlation between egg weight and offspring fitness in the wall brown butterfly *Lasiommata megera*. Oikos, p.376-385.

Karlsson, B., 1987. Variation in egg weight, oviposition rate and reproductive reserves with female age in a natural population of the speckled wood butterfly, *Pararge aegeria*. Ecological Entomology, 12(4), p.473-476.

Kazmer, D.J. and Luck, R.F., 1995. Field tests of the size-fitness hypothesis in the egg parasitoid *Trichogramma pretiosum*. Ecology, 76(2), p.412-425.

Kölliker-Ott, U.M., Blows, M.W. and Hoffmann, A.A., 2003. Are wing size, wing shape and asymmetry related to field fitness of *Trichogramma* egg parasitoids? Oikos, 100(3), p.563-573.

Kimura, K. and Tsubaki, Y., 1985. Egg weight variation associated with female age in *Pieris rapae crucivora* Boisduval (Lepidoptera: Pieridae). Applied Entomology and Zoology, 20(4), p.500-501.

Li, G.Y. and Zhang, Z.Q., 2018. Does size matter? Fecundity and longevity of spider mites (*Tetranychus urticae*) in relation to mating and food availability. Systematic and Applied Acarology, 23(9), p.1796-1808.

Oberhauser, K.S., 1997. Fecundity, lifespan and egg mass in butterflies: effects of male-derived nutrients and female size. Functional Ecology, 11(2), p.166-175.

Parra, J.R.P., Coelho Jr, A., Geremias, L.D., Bertin, A., and Ramos, C.J., 2014. Criação de *Anagasta kuehniella*, em pequena escala, para produção de *Trichogramma*. Occasio, 32.

Ruohomäki, K., Hanhimäki, S. and Haukioja, E., 1993. Effects of egg size, laying order and larval density on performance of *Epirrita autumnata* (Lep., Geometridae). Oikos, p.61-66.

Souza, D. and Faria, L.D.B., 2022. Body size classification of the egg parasitoid *Trichogramma pretiosum* (Hymenoptera: Trichogrammatidae). The Canadian Entomologist, 154, e52, 1-5.

Sagarra, L.A., Vincent, C. and Stewart, R.K., 2001. Body size as an indicator of parasitoid quality in male and female *Anagyrus kamali* (Hymenoptera: Encyrtidae). Bulletin of entomological research, 91(5), p. 363-367.

Steigenga, M.J., Zwaan, B.J., Brakefield, P.M. and Fischer, K., 2005. The evolutionary genetics of egg size plasticity in a butterfly. Journal of Evolutionary Biology, 18(2), p.281-289.

van Lenteren JC (ed) (2003) Quality control and production of biological control agents: theory and testing procedures. CABI Publishing, Wallingford, UK: 265-303.

Vijendravarma, R.K., Narasimha, S. and Kawecki, T.J., 2010. Effects of parental larval diet on egg size and offspring traits in *Drosophila*. Biology letters, 6(2), p.238-241.

Vital, A. Controle biológico se enquadra na ESG e aumenta demanda na utilização de microorganismos. Jornal Cana, 2021. Disponível em: https://jornalcana.com.br/controle-biologico-se-enquadra-na-esg-e-aumenta-demanda-na-utilizacao-de-microorganismos/. Acesso em: 26/01/2023.

Vos, M. and Vet, L.E., 2004. Geographic variation in host acceptance by an insect parasitoid: genotype versus experience. Evolutionary ecology research, 6(7), p.1021-1035.

Wellington, W.G., 1965. Some maternal influences on progeny quality in the western tent caterpillar, *Malacosoma pluviale* (Dyar). The Canadian Entomologist, 97(1), p.1-14.

West, S.A., Flanagan, K.E. and Godfray, H.C.J., 1996. The relationship between parasitoid size and fitness in the field, a study of *Achrysocharoides zwoelferi* (Hymenoptera: Eulophidae). Journal of Animal Ecology, p.631-639.

Wickman, P.O. and Karlsson, B., 1987. Changes in egg color, egg weight and oviposition rate with the number of eggs laid by wild females of the small heath butterfly, *Coenonympha pamphilus*. Ecological Entomology, 12(1), p.109-114.

Wiklund, C. and Persson, A., 1983. Fecundity, and the relation of egg weight variation to offspring fitness in the speckled wood butterfly *Pararge aegeria*, or why don't butterfly females lay more eggs? Oikos, p.53-63.

SEGUNDA PARTE: ARTIGOS

ARTIGO I:

BODY PART SELECTION FOR SIZE CLASSIFICATION OF TWO *TRICHOGRAMMA* SPECIES

Normas do periódico Journal of Applied Entomology (versão preliminar)

Body part selection for size classification of two *Trichogramma* species

Diego de Souza[1*], Geraldo Andrade Carvalho[2], Lucas Del Bianco Faria[3]

[1]*Graduate Program in Entomology, Department of Entomology, Federal University of Lavras, Mailbox 3037, Zip code 37200-900, Lavras, MG, Brazil, https://orcid.org/0000-0001-6759-6919*

[2]*Department of Entomology, Federal University of Lavras, Mailbox 3037, Zip code 37200-900, Lavras, MG, Brazil, https://orcid.org/0000-0002-8379-8008*

[3]*Department of Ecology and Conservation, Institute of Natural Science, Federal University of Lavras, Mailbox 3037, Zip code 37200-900, Lavras, MG, Brazil, https://orcid.org/0000-0002-7230-800X*.

*Author of correspondence: Diego de Souza, desouza.diego@live.com

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ABSTRACT

Body size is an important morphological characteristic that covariates with quality of parasitoids and predators. The actual biography shows that, the bigger the organism, the better are biological parameters and the host location by natural enemies in the field. The standard way of evaluating size of parasitoids of the genus *Trichogramma* is by measuring the tibia but using only one body part to estimate the size of organisms can lead us to misunderstandings. In this paper, commercial *Trichogramma pretiosum* and *Trichogramma galloi* were mounted in slides for microscopy, photographed and the photos were used to measure their antennae, scutellum, ovipositor, tibia and wing. Principal component analysis (PCA) and linear discriminant analysis (LDA) were performed in order to select the best body part that represents their size span. PCA showed that all body parts represent the size span in similar way, and LDA showed that ovipositor is the most representative. We conclude that the best body parts representing the size span of the *Trichogramma* species studied are wing and ovipositor, and at least two body parts are needed to have two sized groups.

Keywords: Quality control, Body Size Span, Egg parasitoid, Host egg.

INTRODUCTION

The body size and appendages are an important morphological characteristic that covariates with physical, behavioral, and history traits of parasitoids and predators (Brown, J.H., Gillooly, J.F., Allen, A.P., Savage, V.M. and West, G.B., 2004; Li and Zhang, 2018). Fecundity, longevity, dispersion, and predation capacity are biological characters direct correlated with the size of the specimens (Berrigan, 1991; Kazmer and Luck, 1995; Sagarra, L.A., Vincent, C. and Stewart, R.K., 2001). The actual biography shows that, the bigger the organism, the better are those biological parameters and studies pointed out that the location of the host in the field is also correlated with the size of the specimens (Clarke and McKenzie, 1992; Kazmer and Luck, 1995; West, S.A., Flanagan, K.E. and Godfray, H.C.J., 1996; Bennett and Hoffmann, 1998; Sagarra et al., 2001; Kölliker-Ott, U.M., Blows, M.W. and Hoffmann, A.A., 2003; Doyon and Boivin, 2005; Durocher-Granger, L., Martel, V. and Boivin, G., 2011). Others biological parameters, like number of eggs in the ovary, egg size, egg viability, reproductive longevity and emergency are all correlated with the size of the specimens (Berrigan, 1991; Kazmer and Luck, 1995; Sagarra et al., 2001; Boivin and Martel, 2012). Thus, all those parameters, including the size of specimens, may be responsible for the higher quality of natural enemies.

The standard way of evaluating quality is mainly through longevity and fecundity, as they are estimates highly related to the efficiency of organisms and are simple to evaluate. Assessing the size of natural enemies is plausible because, in addition to being used as a quality component, it is a simple assessment to perform and is related to several others quality parameters (Van Lenteren, 2003). *Trichogramma pretiosum* Riley, 1879 (Hymenoptera: Trichogrammatidae) and *Trichogramma galloi* Zucchi, 1988 (Hymenoptera: Trichogrammatidae) are the main species utilized as egg parasitoids in Brazil, and widely studied in order to improve their mass rearing processes. *Trichogramma pretiosum* is used to control *Tuta absoluta* Meyrick, 1917 (Lepidoptera: Gelechiidae), *Helicoverpa zea* Boddie, 1850 (Lepidoptera: Noctuidae), *Spodoptera frugiperda* Smith, 1797 (Lepidoptera: Noctuidae), *Anticarsia gemmatalis* Hubner, 1818 (Lepidoptera: Erebidae) and *Chrysodeixis includens* Walker, 1858 (Lepidoptera: Noctuidae), whereas *T. galloi* is used to control *Diatraea saccharalis* Fabricius, 1794 (Lepidoptera: Crambidae). Reared specimen's hind tibia length is a measured of the rearing success. Antennae and ovipositor characteristics are studied to improve the *In Vitro* rearing processes (Cônsoli and Parra, 1996). Small changes in size have a big impact on the flight ability of small insects and, in the case of *T. pretiosum*, wing size can

help predict its fitness in the field, consequently, its ability to control pests (Kölliker-Ott et al., 2003). Therefore, size assessment can contribute to quality control programs in commercial production of natural enemies.

Size evaluation of parasitoids of the genus *Trichogramma* is commonly performed by measuring the wing and tibia (Pavlik, 1993; Kazmer and Luck, 1995; Cônsoli and Parra, 1996; Sorati, M., Newman, M. and Hoffman, A.A., 1996; Bennett and Hoffmann, 1998; Grenier, S., Basso, C. and Pintureau, B., 2001; Kölliker-Ott et al., 2003; Doyon and Boivin, 2005; Durocher-Granger et al., 2011; Reznik and Voinovich, 2016; Souza, D., Monteiro, A.B., and Faria, L.D.B. 2018; Chen, L., Sørensen, J.G. and Enkegaard, A., 2020), but head width (Ishijima, C., Sato, Y. and Ohtaishi, M., 2008), body length (Ahmad, M., Ahmad, M.J., Mishra, R.K. and Sheel, S.K., 2002), body volume (Van Der Woude, E., Smid, H.M., Chittka, L. and Huigens, M.E., 2013) and length of the ovipositor (Grenier et al., 2001) have also been used. While measuring organisms is a technically simple practice, choosing the body part to be measured should be done with caution. Even so, using only one body part to estimate the size of organisms can lead us to misunderstandings. In preliminary work, we classified *T. pretiosum* into small and large specimens based on tibia and wing size. Was not possible to obtain groups with large and small parasitoids based only on one body part. To obtain two distinct groups it is necessary to measure more than one characteristic, since the same insect that is classified as large when using tibia can be classified as small when using wing (Souza and Faria, 2022).

Assuming that the size is an important indicator of the quality of mass rearing commercial parasitoids, and the existence of possible mistakes in the evaluation of the size of these organisms, the objective of this work is to evaluate the size span of commercial *T. pretiosum* and *T. galloi* by classifying them into size categories and determining the best body parts to represent body size.

MATERIAL AND METHODS

Parasitoid size measurement

Parasitoids used in the experiment were offered by Koppert Biological Systems® from Brazil. The commercial products “Pretiobug®” and “Galloibug®”, composed by *T. pretiosum* and *T. galloi*, respectively, were received as pupae inside of *Ephestia kuehniella* Zeller, 1879 (Lepidoptera: Pyralidae) eggs. For each specie, 600 parasitized *E. kuehniella* eggs were selected and maintained in an acclimated room at 25ºC and 60% RH until adult emergency.

The adults were kept in the Eppendorf® microtubes with 70% alcohol until triage for body size measurement. Two hundred thirty six *T.pretiosum* females were measured, and they were prepared submerging in potassium hydroxide 10% during 5 to 15 minutes to clarify the insect tissues, then, they were washed with distilled water (Querino and Zucchi, 2011). Females were mounted using Hoyer's medium in ventro/dorsal position on slides for microscopy. Slides were photographed using a digital camera AmScope MU1003 connected to a binocular microscope OLYMPUS BX40, and the photos were used to measure the following body parts: antenna, from the base to the top of the first flagellomere (Fig 1A); scutellum, measured by the distance between the anterior and posterior left bristles (Fig 1B); ovipositor length, measured by the most inner to the farther extreme (Fig 1C); tibia length, measured from the junction of the femur with the tibia to the junction of the tibia with the tarsus (Fig 1D); wing was measured in two different ways, the first is the standard method used for *Trichogramma* species, measured from the beginning to the end of the MA vein (Fig 1E), the second is a new approach, measured the distance among the three main bristles in the marginal vein (Fig. 1F).

Statistical analysis

Data normality was accessed by applying Shapiro-Wilk's test and presence of outliers was checked plotting Cleveland dot plot. Correlations between the body parts measured was assessed by using the Pearson correlation coefficient. Principal Component Analysis (PCA) was performed to find the very best body part that represents the most variation in body size. Hereafter, each of those body parts were classified based on deciles using their size span and fourteen groups with small and large individuals were created combining them (Table 1). Small specimens belong to the 1st to 3rd deciles, and large specimens belong to the 8th to 10th deciles of the size span (Fig. 2). Linear Discriminant Analysis (LDA) was performed on groups to evaluate the distinguishability among them. All analyzes were performed using R, version 4.2.2 (R Core Team 2022).

RESULTS

For *T. pretiosum*, the variables antenna, tibia and wing were not normally distributed. Scutellum, ovipositor length and marginal vein were normally distributed. For *T. galloi*, antenna, scutellum, tibia and wing were not normally distributed. Ovipositor and marginal vein were normally distributed (Table 2). Outliers observed (data not shown) in the populations of either *T. pretiosum* and *T. galloi* were insects with malformation, poor mounted and siblings (two parasitoids from the same egg). They all were removed from posterior analysis. The most

correlated variables in *T. pretiosum* were tibia and wing ($r = 0.87$; $P < 0.05$), antenna and tibia ($r = 0.75$; $P < 0.05$), ovipositor and tibia ($r = 0.75$; $P < 0.05$), antenna and wing ($r = 0.72$; $P < 0.05$), and wing measurements ($r = 0.68$; $P < 0.05$). For *T. galloi*, ovipositor and wing ($r = 0.81$; $P < 0.05$) were the most correlated, followed by scutellum and ovipositor ($r = 0.77$; $P < 0.05$), antenna and tibia ($r = 0.75$; $P < 0.05$), and wing measurements ($r = 0.73$; $P < 0.05$).

The first (PC1) and second (PC2) principal components from the principal component analysis explained 59.08% and 19.17% of the variability in size span, respectively (Figure 3 – Scree plot). In the PC1, which is a principal component that represents the size of *T. pretiosum* and *T. galloi*, wing length was the major contributor to explain the size variation, followed by tibia length and marginal vein (Table 3). In the PC2, which is related to the body form of specimens, scutellum was the most import body part, followed by ovipositor length (Table 3). Those two body parts are related to the large specimens, whereas antenna and tibia length are related to the small specimens (Figure 4 - PCA), PC3 and PC4 account for 9.50% and 5.42% of body size variation, respectively. In the PC3, whereas marginal vein is the most important body party and is related to large specimens, ovipositor is the second most important and is related to small specimens. For PC4, scutellum is related to small specimens, whereas ovipositor is related to large specimens. Together, the four principal components account for 93% of the size variation of body parts in *T. pretiosum* and *T. galloi*. The weight of the body parts on each principal component combined with the correlations among them lead us to consider the wing, tibia and ovipositor lengths as the most important body parts related to body size variation in *T. pretiosum* and *T. galloi*.

Hereafter, linear discriminant analysis was performed for each specie (Table 4 and Figure 5 to 10) using the groups created as shown in the Table 1. Two hundred thirty-six *T. pretiosum* specimens were used to create the groups and as group creation occurs, some specimens were classified in more than one group, yielding more observations for the LDA than the number of specimens itself – for example, a specimen could be classified as small when using tibia, but large when using wing (Souza and Faria, 2022).

The first linear discriminant analysis - LDA1 (Figure 5) accounts for specimens classified using only one body part at a time, having six groups (G1, G2, T1, T2, WW1, WW2 – Table 1) and 423 observations. The first linear discriminant function accounts for 96% of the proportion of trace, and the most important body part to separate the groups was the ovipositor length (Table 4). The second linear discriminant analysis - LDA2 (Figure 6) accounts for specimens classified using two body parts, having six groups (GT1, GT2, GWW1, GWW2,

TWW1, TWW2 – Table 1) and 312 observations. The first linear discriminant function accounts for 99% of the proportion of trace, and the most important body part to separate the groups was the ovipositor length (Table 4). The third linear discriminant analysis - LDA3 (Figure 7) was performed comparing the best combinations of body parts with a group created using the three body parts at the same time. Ten groups (G1, G2, GT1, GT2, GTWW1, GTWW2, GWW1, GWW2, TWW1, TWW2 – Table 1) and 541 observations were compared. The first linear discriminant function accounts for 98% of the proportion of trace, and again, the most important body part to separate the groups was the ovipositor length (Table 4).

Two hundred forty-eight *T. galloi* specimens were used to create the groups, and like for *T. pretiosum*, as the group creation occurs, some specimens were classified in more than one group. The fourth linear discriminant analysis – LDA4 (Figure 8) accounts for specimens classified using only one body part at a time, having six groups and 444 observations. The first linear discriminant function accounts for 70% of the proportion of trace, and the most important body part to separate the groups was the ovipositor length (Table 4). The fifth linear discriminant analysis LDA5 (Figure 9) accounts for specimens classified using two body parts, having six groups and 226 observations. The first linear discriminant function accounts for 84% of the proportion of trace, and the most important body part to separate the groups was the ovipositor length (Table 4). The sixth and last linear discriminant analysis LDA 6 (Figure 10) was performed comparing the best combinations of body parts with a group created using the three body parts at the same time. Ten groups and 420 observations were compared. The first linear discriminant function accounts for 89% of the proportion of trace, and again, the most important body part to separate the groups was the ovipositor length (Table 4).

DISCUSSION

The best body parts to represent the size span of *T. pretiosum* and *T. galloi* were ovipositor and wing, and three things were considered to achieve this conclusion, the representability of size span, indicated in the PCA; the biological importance of the body part (e.g. - flying, walking, laying egg); and the capability to separate in small and large specimens, indicated in the LDA.

The results of principal component analysis indicate that all body parts represent the size span in similar way, thus, is impossible to select just one body part to represent the size span of *T. pretiosum* and *T. galloi*. Tibia, marginal vein, and wing represents the general size span, whereas ovipositor and scutellum are related to variations in body form in large

specimens, and tibia and antenna in small specimens. The measurement feasibility and the biological function were considered to choose among those body parts to follow the analysis.

Tibia is the easiest to measure and the most common body part used to assess size of *Trichogramma* species. Tibia is a part of the leg of the insect and *Trichogramma* spp. use walking to measure and select host eggs (Schmidt and Smith, 1987). During the wasp's examination walk the female is able to determine the host volume by assessing the relative surface curvature and the exposed surface area. Despite the capacity of large and small females to evaluate host size did not differ, and walking speed being independent of body size, is the walking that provide the parasitoid the means to assess the host volume, thus tibia is an important body part to measure. Ovipositor is also easy to measure, and despite the need to clarify the insect tissues before measurements, it is very reliable. *Trichogramma* species are egg parasitoids, and the ovipositor has the function of laying the parasitoid's egg inside the host egg. Moreover, the size of the ovipositor could be an indicator of superparasitism, because it varies according to the number of adults developed inside the same host (Grenier et al., 2001). Wing is an important body part because it is related to the flying capacity. Egg parasitoids need to fly in order to seek for host and move in the ambient and wing size was related to the female's ability to search and parasitize host eggs in the field (Kazmer and Luck, 1995; Bennett and Hoffmann, 1998; Fournier and Boivin, 2000). Females with extremely large wing had the highest host field location (Bennett and Hoffmann, 1998). Thus, wing should be chosen to measure the parasitoid size when relating it with biological quality parameters, especially in the applied perspective as wing size is an important characteristic determining the effectiveness of egg parasitoids in biological control (Kazmer and Luck, 1995).

In terms of measurement feasibility, antenna, tibia, ovipositor, and marginal vein are the easiest body parts to measure, but attention is need doing it. During the clarifying process some deformations could occur, for example, antennae could writhe and shrink, making unfeasible the measurement. Wing and scutellum are good traits to measure, but both need to be done carefully. As wings are made of very softy tissue, they sometimes fold and distort during slide mounting process. In a similar way, scutellum could break off if the insect is clarified too much or the coverslip is hardly press on the insect. Although marginal vein is easy and reliable to measure, and it is correlated with wing size, we choose to follow the analysis using the wing length because its importance in flying and it is more mentioned in the biography (Kazmer and Luck, 1995; Bennett and Hoffmann, 1998).

Therefore, considering the biological importance of the body parts and the results of principal component analysis, the most interesting body parts to measure are tibia, ovipositor, and wing. Following with discriminant analysis, tibia does not seem to be a god choice as it has a high variability among the groups. For *T.preiosum*, specimens with large tibias (T2) could also have small ovipositor (G1), and even when selecting large tibia and wing together (TWW2) we could confuse with specimens with small ovipositor. This confound effect was also observed in preliminary work, when just wing and tibia were measured and was observed that the specimen classified as large by its wing size was also classified as small by its tibia size (Souza and Faria, 2022). For *T. galloi* the relationship for tibia with small and large specimens is not clear. When using just one body part in the size classification, specimens classified as large by either ovipositor or wing could have small or large tibias. When using two body parts, groups that have tibia size as criterion of selection tends to overlap groups with small ovipositor and small wing. Specimens classified as small by ovipositor and wing are close related to specimens classified as large by tibia and wing.

For both species, the ovipositor length was the best body part to use and have the highest coefficient of linear discriminant, but groups based on only it could still be confounded with groups based on tibia and wing. Therefore, to have two reliable separated groups based on size span, at least two body parts need to be measured. The proportion of trace is the highest for *T. galloi* when using three body parts, but good separability is achieved when using two body parts. For *T. pretiosum*, the highest proportion of trace is achieved using two body parts, and considering the coefficients of discriminant analysis, ovipositor and wing are the most important body parts to separate the groups in the linear discriminant analysis. Thus, measuring ovipositor and wing are the best way to separate both species in groups of small and large specimens.

As far as we know, that was the first time that a *Trichogramma* spp. body size classification was studied using different body parts. The approach used here highlight the importance of knowing the behavior and role of the species in order to select the best body parts to study. In conclusion, to have two sized specimens' populations the best body parts to measure are ovipositor and wing. Future research should evaluate the quality parameters of large specimens selected based on ovipositor and wing length and investigate the factors that affect the size variation of parasitoids reared on standardized resources.

Conflict of Interest: none.

REFERENCES

Ahmad, M., Ahmad, M.J., Mishra, R.K. and Sheel, S.K., 2002. Superparasitism by *Trichogramma poliae* in the eggs of *Clostera cupreata* (Lepidoptera: Notodontidae) and its effect on offspring. Journal of Tropical Forest Science, p.61-70.

Bennett, D.M. and Hoffmann, A.A., 1998. Effects of size and fluctuating asymmetry on field fitness of the parasitoid *Trichogramma carverae* (Hymenoptera: Trichogrammatidae). Journal of Animal Ecology, p.580-591.

Berrigan, D., 1991. The allometry of egg size and number in insects. *Oikos*, p.313-321. DOI: 10.2307/3545073

Boivin, G. and Martel, V., 2012. Size-induced reproductive constraints in an egg parasitoid. Journal of Insect Physiology, 58(12), p.1694-1700. DOI: 10.1016/j.jinsphys.2012.10.014

Brown, J.H., Gillooly, J.F., Allen, A.P., Savage, V.M. and West, G.B., 2004. Toward a metabolic theory of ecology. Ecology, 85(7), p.1771-1789. DOI: 10.1890/03-9000

Clarke, G.M. and McKenzie, L.J., 1992. Fluctuating asymmetry as a quality control indicator for insect mass rearing processes. Journal of Economic Entomology, 85(6), p.2045-2050. DOI: 10.1093/jee/85.6.2045

Cônsoli, F.L. and Parra, J.R.P., 1996. Biology of *Trichogramma galloi* and *T. pretiosum* (Hymenoptera: Trichogrammatidae) reared in vitro and in vivo. Annals of the Entomological Society of America, 89(6), p.828-834. DOI: 10.1093/aesa/89.6.828

Chen, L., Sørensen, J.G. and Enkegaard, A., 2020. Effects of temperature on development, reproduction and size of *Trichogramma achaeae*: Implications for biological control. bioRxiv. DOI: 10.1101/2020.12.14.422618

Doyon, J. and Boivin, G., 2005. The effect of development time on the fitness of female *Trichogramma evanescens*. Journal of Insect Science, 5(1), 4. DOI: 10.1093/jis/5.1.4

Durocher-Granger, L., Martel, V. and Boivin, G., 2011. Gamete number and size correlate with adult size in the egg parasitoid *Trichogramma euproctidis*. Entomologia Experimentalis et Applicata, 140(3), p.262-268. DOI: 10.1111/j.1570-7458.2011.01158.x

Fournier, F. and Boivin, G., 2000. Comparative dispersal of *Trichogramma evanescens* and *Trichogramma pretiosum* (Hymenoptera: Trichogrammatidae) in relation to environmental conditions. Environmental Entomology, 29(1), p.55-63. DOI: 10.1603/0046-225X-29.1.55

Grenier, S., Basso, C. and Pintureau, B., 2001. Effects of the host species and the number of parasitoids per host on the size of some Trichogramma species (Hymenoptera: Trichogrammatidae). Biocontrol Science and Technology, 11(1), p.21-26. DOI: 10.1080/09583150020029709

Ishijima, C., Sato, Y. and Ohtaishi, M., 2008. Effect of temperature and host on the development, sex ratio, emergence rate and body size of *Trichogramma dendrolimi* Matsumura (Hymenoptcra: Trichogrammatidae), an egg parasitoid of the tea tortrix. Japanese Journal of Applied Entomology and Zoology, 52(4), p.193-200.

Kazmer, D.J. and Luck, R.F., 1995. Field tests of the size-fitness hypothesis in the egg parasitoid *Trichogramma pretiosum*. Ecology, 76(2), p.412-425. DOI: 10.2307/1941200

Kölliker-Ott, U.M., Blows, M.W. and Hoffmann, A.A., 2003. Are wing size, wing shape and asymmetry related to field fitness of *Trichogramma* egg parasitoids?. Oikos, 100(3), p.563-573. DOI: 10.1034/j.1600-0706.2003.12063.x

Li, G.Y. and Zhang, Z.Q., 2018. Does size matter? Fecundity and longevity of spider mites (*Tetranychus urticae*) in relation to mating and food availability. Systematic and Applied Acarology, 23(9), p.1796-1808. DOI: 10.11158/saa.23.9.6

Pavlik, J., 1993. The size of the female and quality assessment of mass-reared *Trichogramma* spp. Entomologia Experimentalis et Applicata, 66(2), p .171-177. DOI: 10.1111/j.1570-7458.1993.tb00705.x

Querino, R. B. and Zucchi, R. A., 2011. Guia de identificação de *Trichogramma* para o Brasil. Embrapa Informação Tecnológica, 103 p. : il.

R Core Team. 2022. R: a language and environment for statistical computing. Available from https://www.r-project.org/ [accessed 1 November 2022]

Reznik, S.Y. and Voinovich, N.D., 2016. Emergence rhythm and quality control in the laboratory and mass rearing of *Trichogramma* species (Hymenoptera: Trichogrammatidae). Entomological Review, 96(8), p.965-973. DOI: 10.1134/S0013873816080017

Sagarra, L.A., Vincent, C. and Stewart, R.K., 2001. Body size as an indicator of parasitoid quality in male and female *Anagyrus kamali* (Hymenoptera: Encyrtidae). Bulletin of entomological research, 91(5), p. 363-367. DOI: 10.1079/BER2001121

Schmidt, J.M. and Smith, J.J.B., 1987. The measurement of exposed host volume by the parasitoid wasp *Trichogramma minutum* and the effects of wasp size. Canadian Journal of Zoology, 65(12), p.2837-2845. DOI: 10.1139/z87-432

Sorati, M., Newman, M. and Hoffman, A.A., 1996. Inbreeding and incompatibility in *Trichogramma* nr. *brassicae*: evidence and implications for quality control. Entomologia Experimentalis et Applicata, 78(3), p.283-290. DOI: 10.1111/j.1570-7458.1996.tb00792.x

Souza, D., Monteiro, A.B., and Faria, L.D.B. 2018. Morphometry, allometry, and fluctuating asymmetry of egg parasitoid *Trichogramma pretiosum* under insecticide influence. Entomologia Experimentalis et Applicata, 166: 298–303. DOI: 10.1111/eea.12665

Souza, D. and Faria, L.D.B., 2022. Body size classification of the egg parasitoid *Trichogramma pretiosum* (Hymenoptera: Trichogrammatidae). The Canadian Entomologist, 154, e52, 1-5. DOI: 10.4039/tce.2022.40

Van Der Woude, E., Smid, H.M., Chittka, L. and Huigens, M.E., 2013. Breaking Haller's rule: brain-body size isometry in a minute parasitic wasp. Brain, Behavior and Evolution, 81(2), p.86-92. DOI: 10.1159/000345945

van Lenteren JC (ed) (2003) Quality control and production of biological control agents: theory and testing procedures. CABI Publishing, Wallingford, UK: 265-303.

West, S.A., Flanagan, K.E. and Godfray, H.C.J., 1996. The relationship between parasitoid size and fitness in the field, a study of *Achrysocharoides zwoelferi* (Hymenoptera: Eulophidae). Journal of Animal Ecology, p.631-639. DOI: 10.2307/5742

Table 1. Groups created based on body correlations and results from the principal component analysis.

Group	Description	Number of specimens	
		Trichogramma pretiosum	*Trichogramma galloi*
G1	Small specimens based on ovipositor length	72	75
G2	Large specimens based on ovipositor length	69	73
T1	Small specimens based on tibia length	72	75
T2	Large specimens based on tibia length	69	73
WW1	Small specimens based on wing length	72	75
WW2	Large specimens based on wing length	69	73
GT1	Small specimens based on ovipositor and tibia lengths	53	38
GT2	Large specimens based on ovipositor and tibia lengths	48	17
GWW1	Small specimens based on ovipositor and wing lengths	50	53
GWW2	Large specimens based on ovipositor and wing lengths	47	53
TWW1	Small specimens based on tibia and wing lengths	62	40
TWW2	Large specimens based on tibia and wing lengths	52	25
GTWW1	Small specimens based on ovipositor, tibia, and wing lengths	47	36
GTWW2	Large specimens based on ovipositor, tibia, and wing lengths	41	10

Table 2. Maximum, minimum, and mean size (millimeters) measurements for each body part and specie with standard deviation, coefficient of variation and Shapiro's Will test significance.

Body part	Max.	Min.	Mean	SD	CV(%)	Shapiro.test (W)	Shapiro.test (*p*)	*n*
Trichogramma pretiosum								236
Antenna length	0.09044	0.07018	0.08201	0.003910796	4.768743	0.9737	< 0.05	
Scutellum	0.04567	0.02854	0.03646	0.003444113	9.44701	0.98976	0.07	
Ovipositor length	0.1665	0.1234	0.1446	0.007442449	5.147098	0.99445	0.40	
Tibia length	0.1775	0.1314	0.1596	0.008893428	5.572277	0.97043	< 0.05	
Marginal vein	0.04989	0.03366	0.04152	0.003054441	7.355913	0.99524	0.57	
Wing length	0.2608	0.1855	0.2336	0.01269567	5.435765	0.97011	< 0.05	
Trichogramma galloi								248
Antenna length	0.09016	0.0665	0.08122	0.003853771	4.744984	0.9643	< 0.05	
Scutellum	0.04675	0.02794	0.03635	0.004366996	12.01473	0.93745	< 0.05	
Ovipositor length	0.1861	0.1235	0.1556	0.01131996	7.274241	0.99398	0.32	
Tibia length	0.1721	0.1237	0.1553	0.00868169	5.590932	0.96277	< 0.05	
Marginal vein	0.04665	0.03249	0.03987	0.002695829	6.761839	0.99393	0.33	
Wing length	0.2560	0.1901	0.2294	0.01276519	5.564593	0.97778	< 0.05	

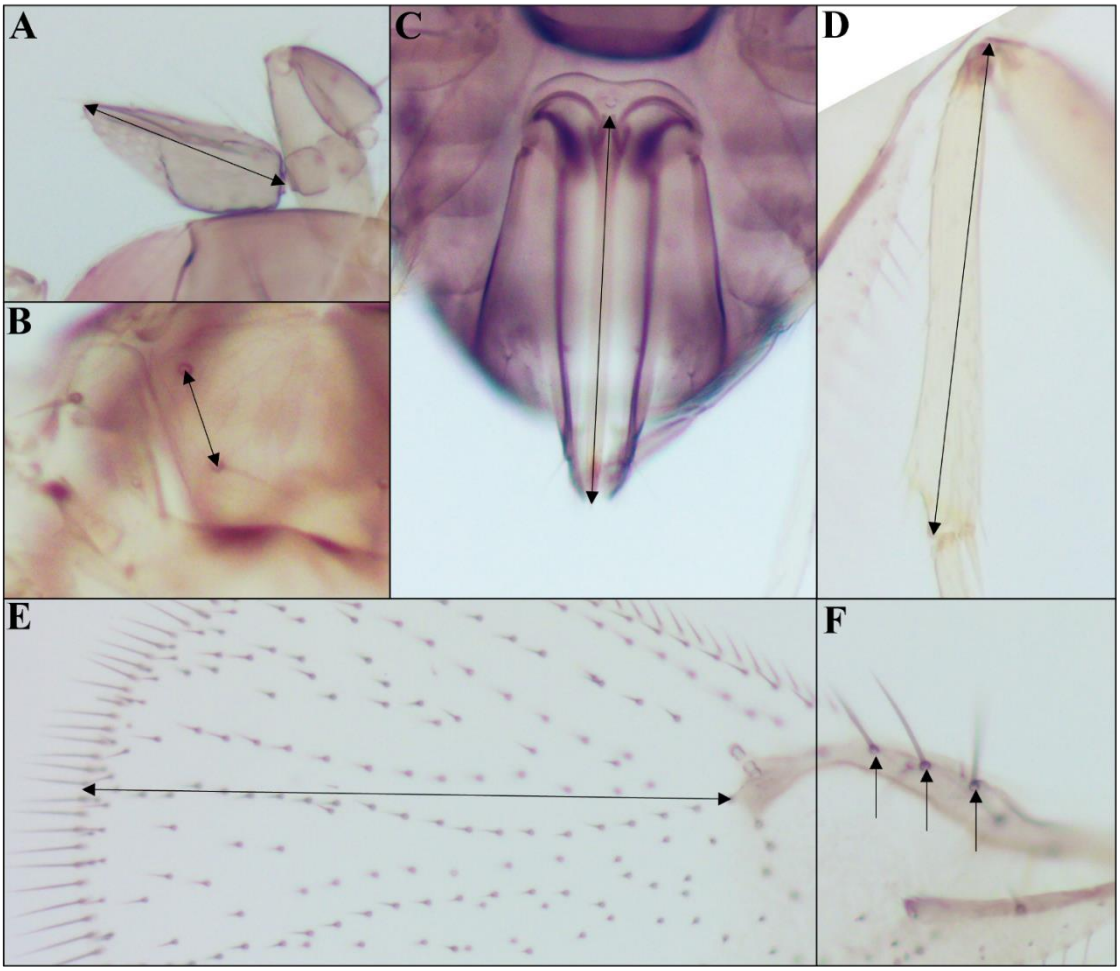
Table 3. Principal Component Analysis for all body parts measured for *Trichogramma pretiosum* and *Trichogramma galloi*.

Body parts	PC1	PC2	PC3	PC4
Antenna	0.3948083	-0.44256720	-0.4033270	-0.29406278
Scutellum	0.3545710	0.57538677	0.1995342	-0.65318723
Ovipositor	0.3421933	0.52277029	-0.5529442	0.53330031
Tibia	0.4252964	-0.43309163	-0.1421312	-0.03222520
Marginal vein	0.4197598	-0.10329527	0.6781183	0.44840927
Wing	0.4942006	0.03920822	0.1082611	-0.01884067
Standard deviation	1.8828	1.0726	0.75558	0.57033
Proportion of variance	0.5908	0.1917	0.09515	0.05421
Cumulative proportion	0.5908	0.7826	0.87773	0.93194

Table 4. Coefficients of linear discriminant analysis and proportion of trace for the six discriminant analysis performed for *Trichogramma pretiosum* (LDA1, LDA2, LDA3) and *Trichogramma galloi* (LDA4, LDA5, LDA6) for all body parts measured.

Discriminant analysis	Coefficients of linear discriminants						Proportion of trace
	Antenna	Scutellum	Ovipositor	Tibia	Marginal vein	Wing	
LDA1	-21.48296	30.23059	89.00836	46.58372	-34.15672	55.90114	96.34%
LDA2	-34.23100	43.30953	95.94818	62.69721	-43.03895	68.55543	99.33%
LDA3	-34.19472	20.27794	143.18242	40.46925	-44.47194	57.80616	98.68%
LDA4	-37.541987	34.756205	66.438994	-3.573123	26.982901	35.759359	70.14%
LDA5	-70.34443	59.44306	107.89817	-18.42924	25.05668	60.72364	84.01%
LDA6	-70.22834	64.10724	119.03804	-13.25807	28.39509	45.49194	89.57%

Figure 1



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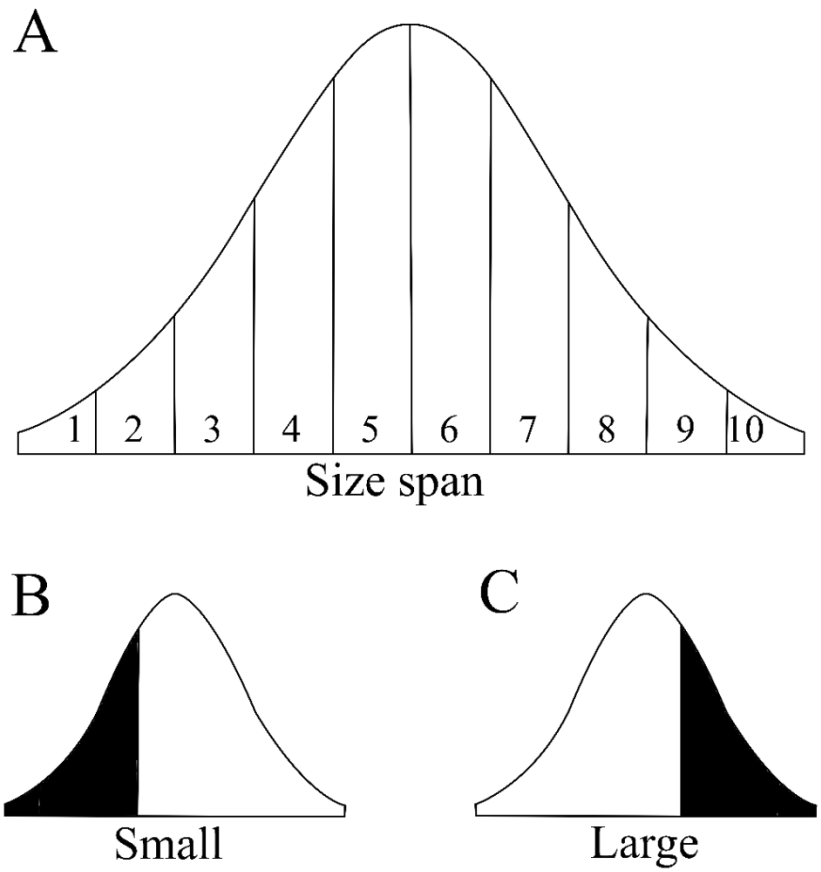

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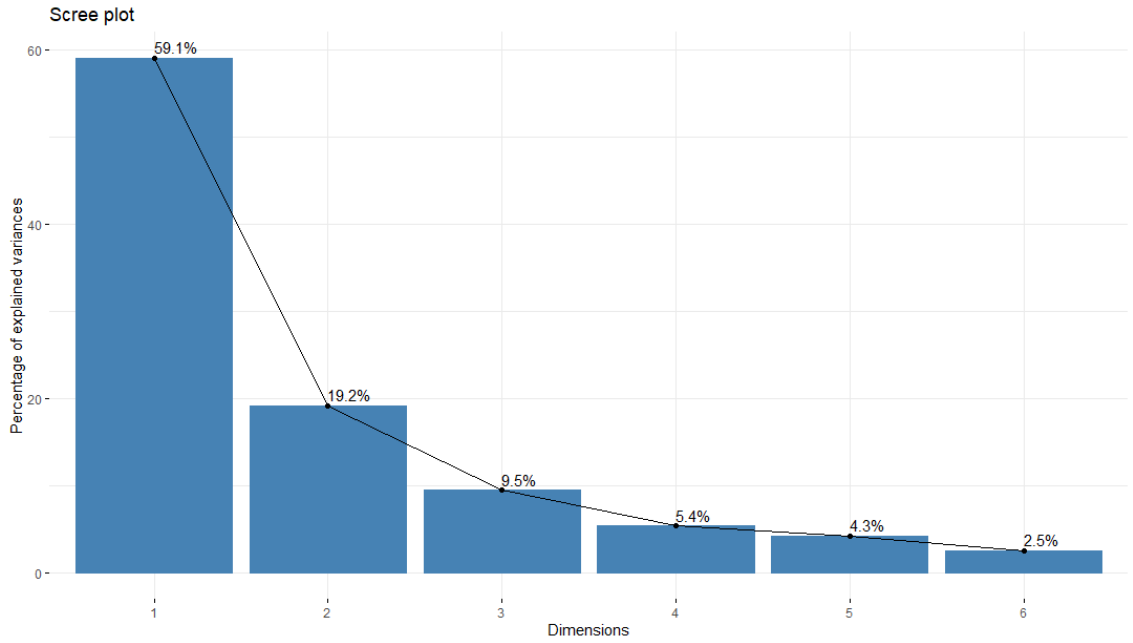
Scree plot
Percentage of explained variances
60
40
20
0
59.1%
19.2%
9.5%
5.4%
4.3%
2.5%
1
2
3
4
5
6
Dimensions

Figure 4

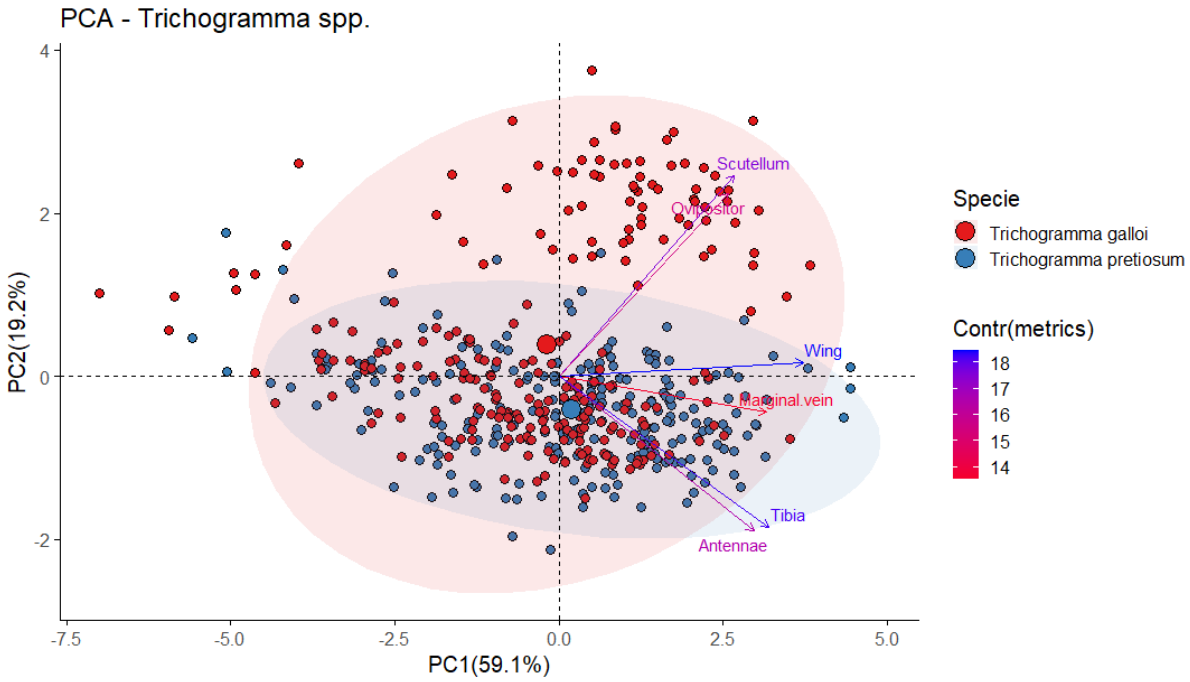
PCA - Trichogramma spp.
PC2(19.2%)
PC1(59.1%)
4
2
0
-2
-7.5
-5.0
-2.5
0.0
2.5
5.0
Scutellum
Ovipositor
Wing
Marginal.vein
Tibia
Antennae
Specie
Trichogramma galloi
Trichogramma pretiosum
Contr(metrics)
18
17
16
15
14

Figure 5

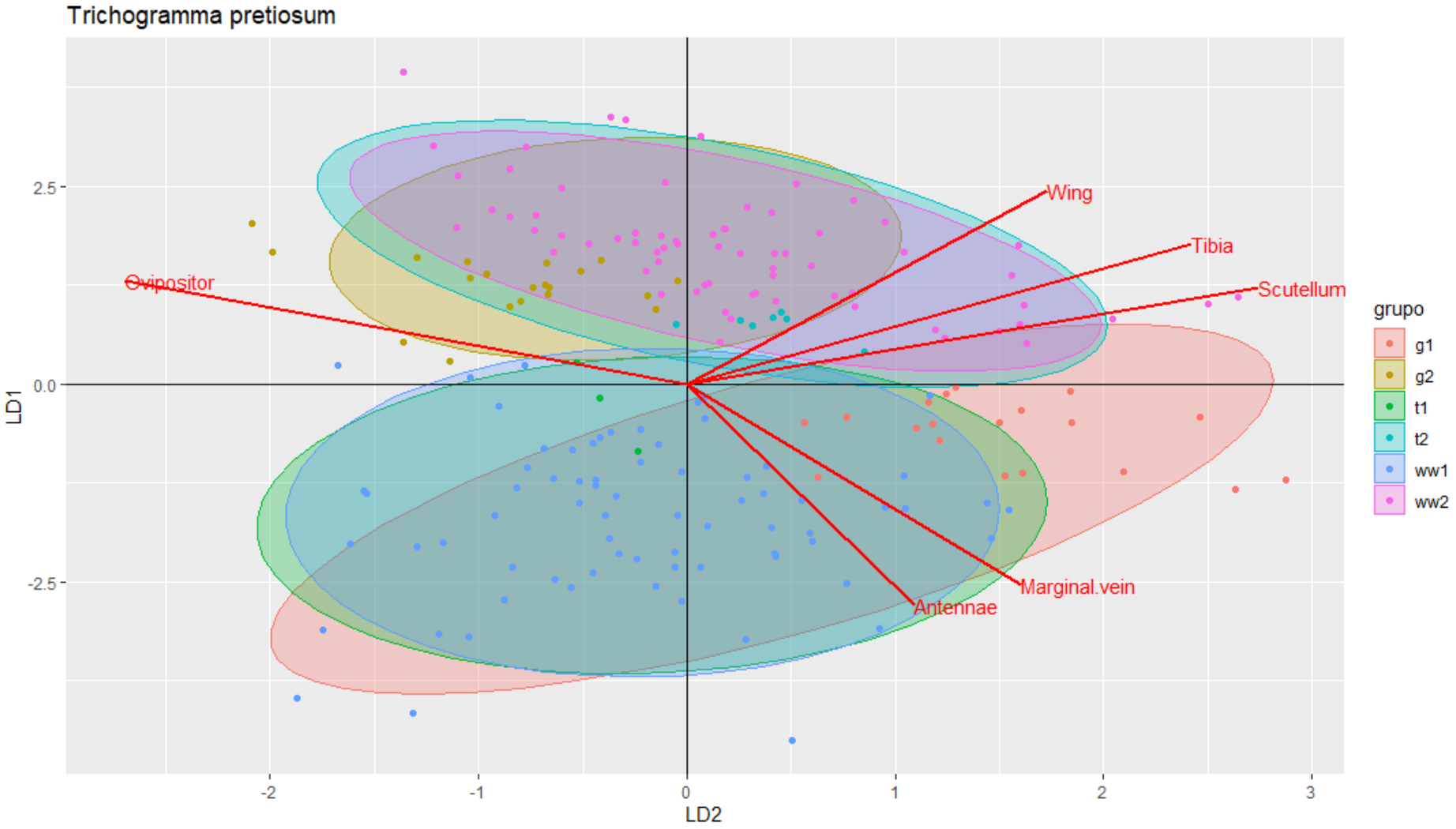
Trichogramma pretiosum
Wing
Tibia
Scutellum
Ovipositor
Marginal.vein
Antennae
LD1
LD2
2.5
0.0
-2.5
-2
-1
0
1
2
3
grupo
g1
g2
t1
t2
ww1
ww2

Figure 6

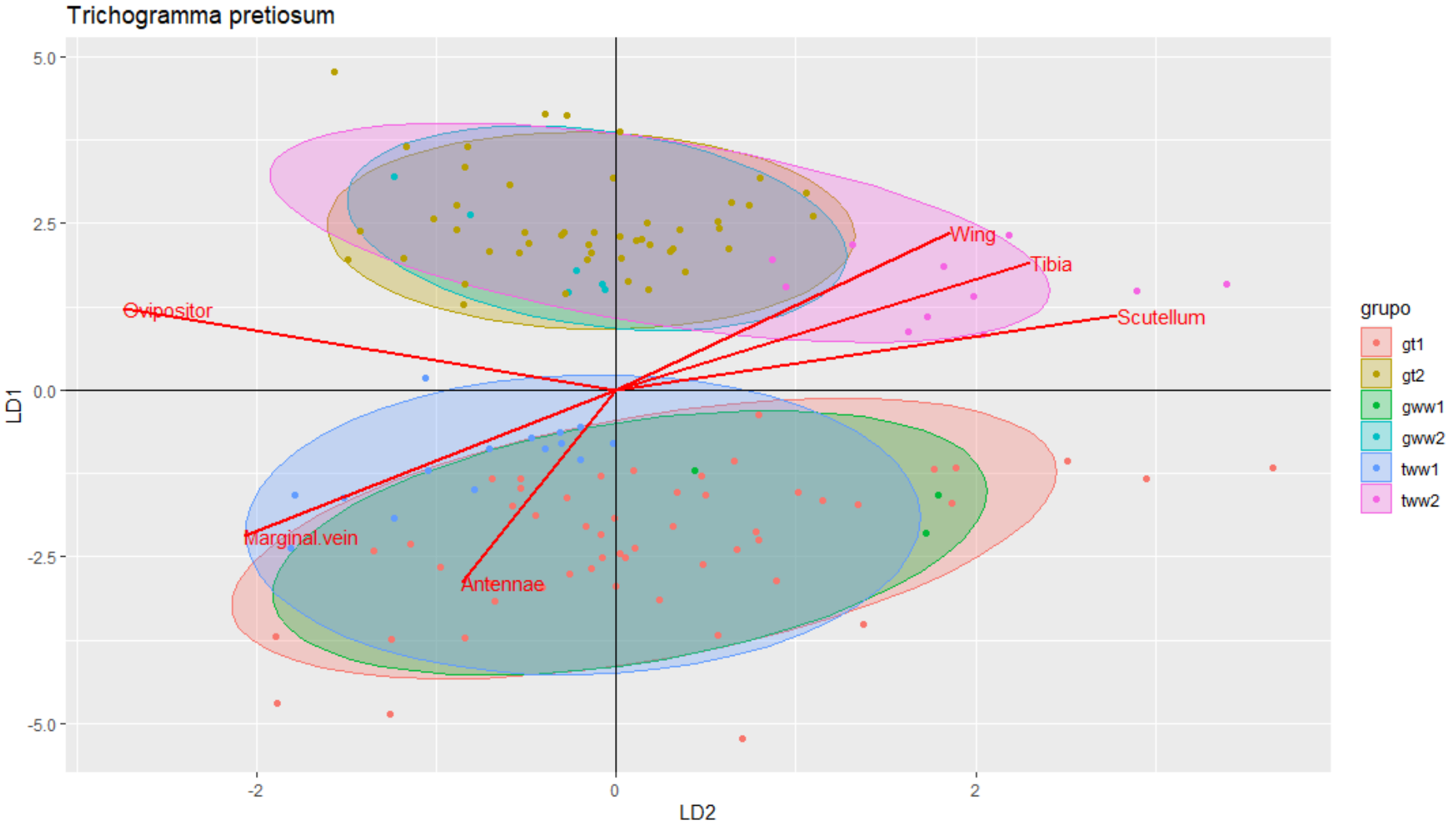
Trichogramma pretiosum
5.0
2.5
0.0
-2.5
-5.0
LD1
-2
0
2
LD2
Wing
Tibia
Scutellum
Ovipositor
Marginal.vein
Antennae
grupo
gt1
gt2
gww1
gww2
tww1
tww2

Figure 7

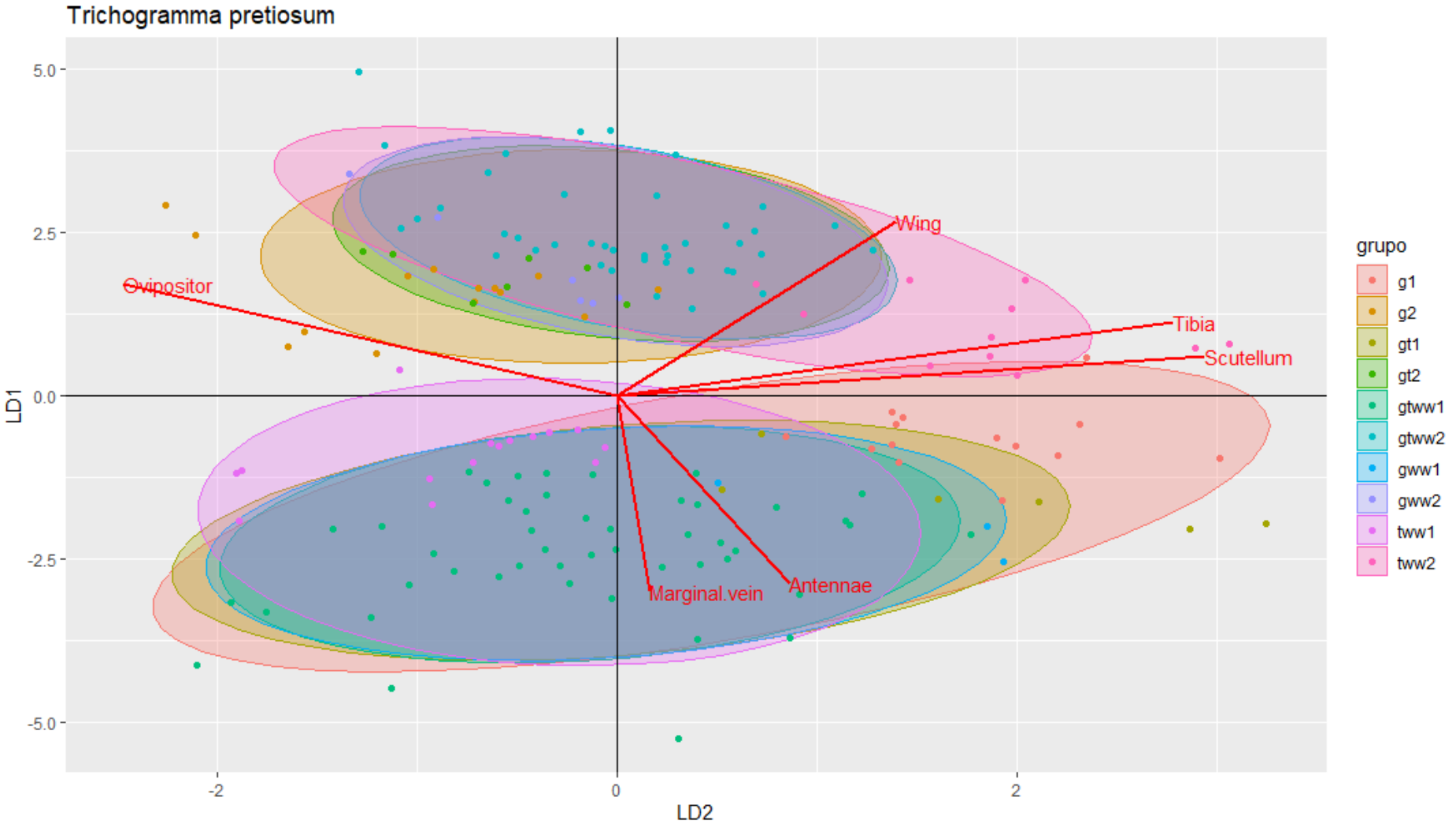
Trichogramma pretiosum
5.0
2.5
0.0
-2.5
-5.0
LD1
-2
0
2
LD2
Wing
Ovipositor
Tibia
Scutellum
Marginal.vein
Antennae
grupo
g1
g2
gt1
gt2
gtww1
gtww2
gww1
gww2
tww1
tww2

Figure 8

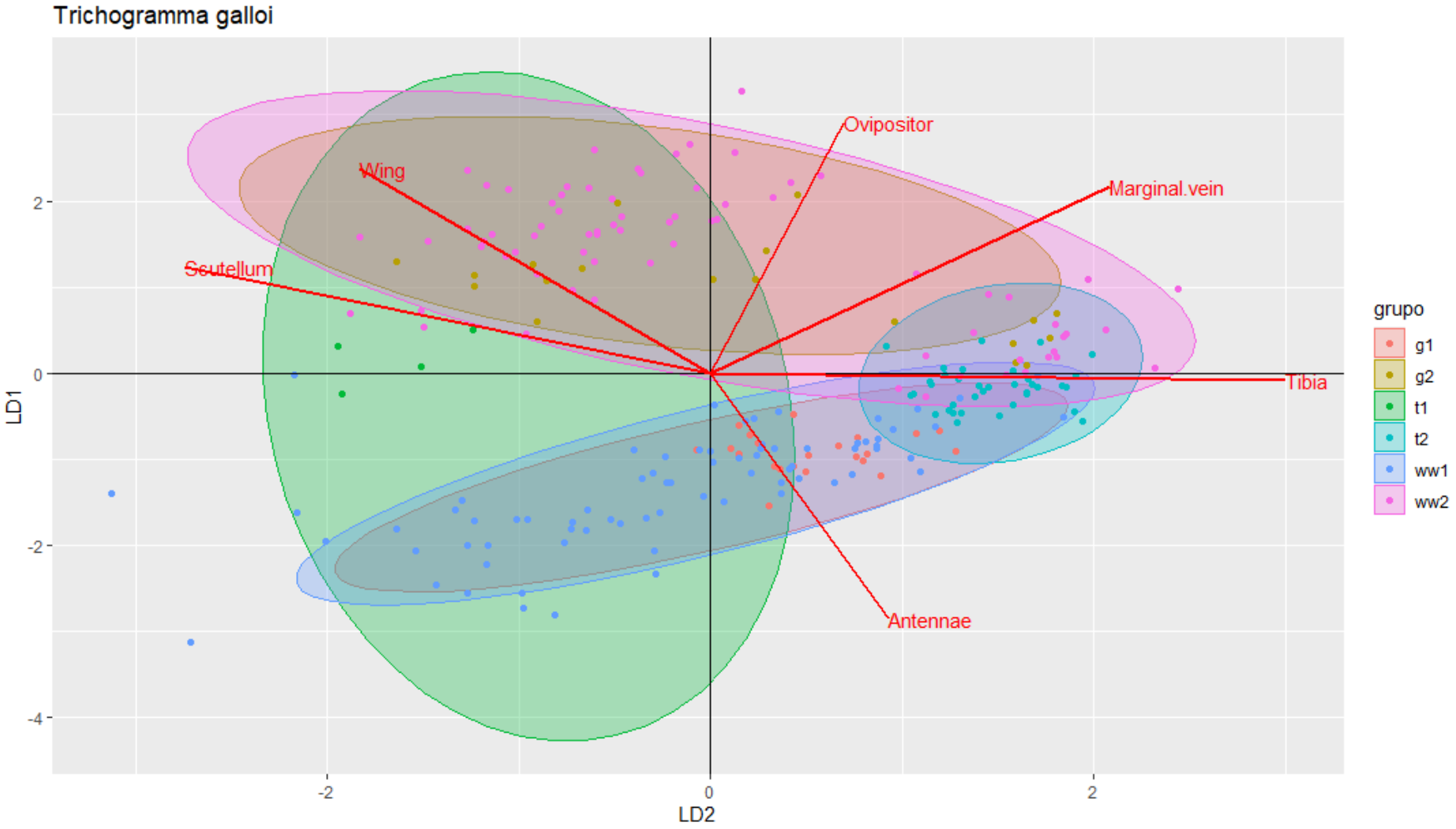
Trichogramma galloi
Ovipositor
Wing
Marginal.vein
Scutellum
Tibia
Antennae
LD1
LD2
2
0
-2
-4
-2
0
2
grupo
g1
g2
t1
t2
ww1
ww2

Figure 9

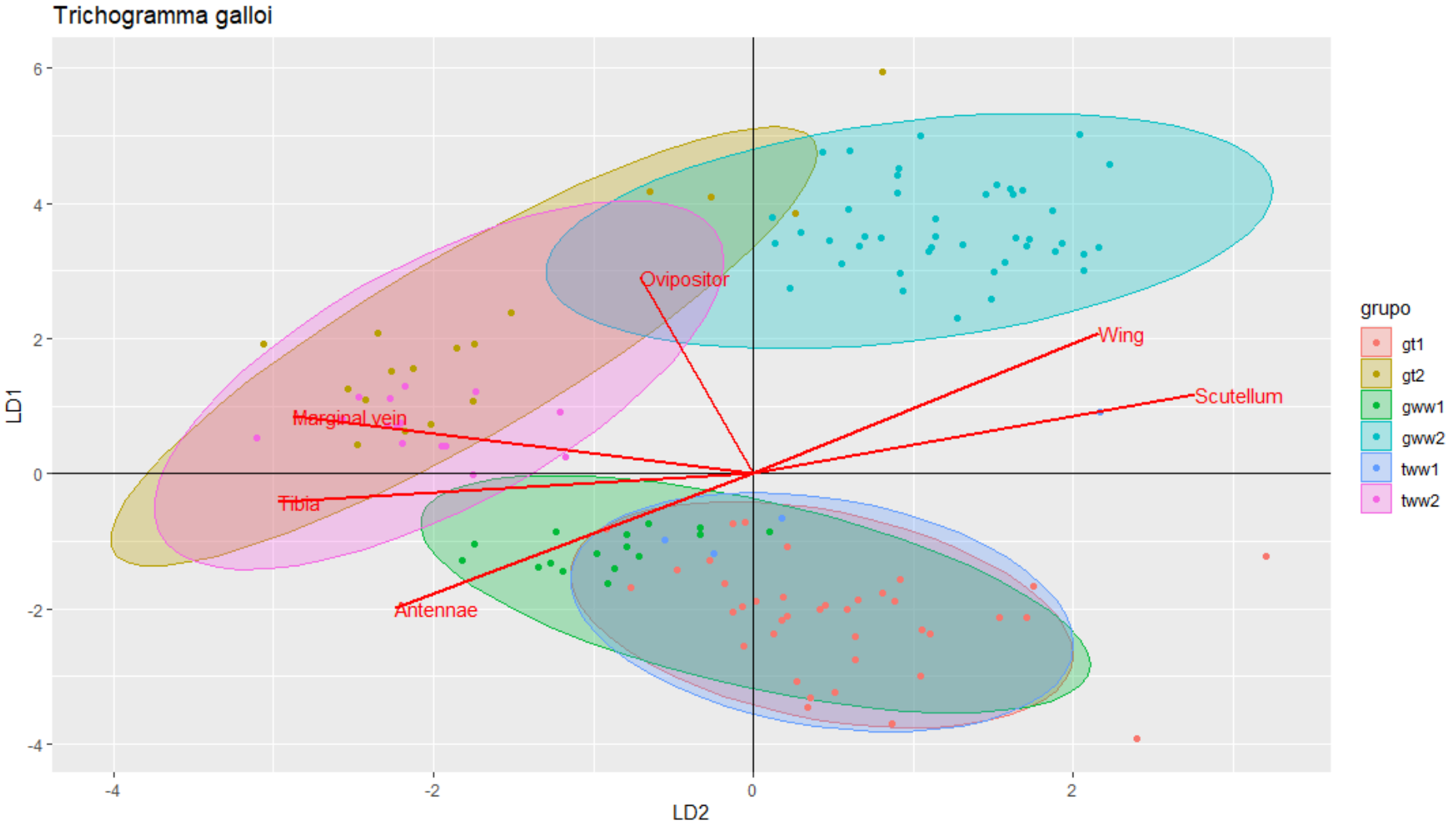
Trichogramma galloi
LD1
LD2
6
4
2
0
-2
-4
-4
-2
0
2
Ovipositor
Wing
Scutellum
Marginal vein
Tibia
Antennae
grupo
gt1
gt2
gww1
gww2
tww1
tww2

Figure 10

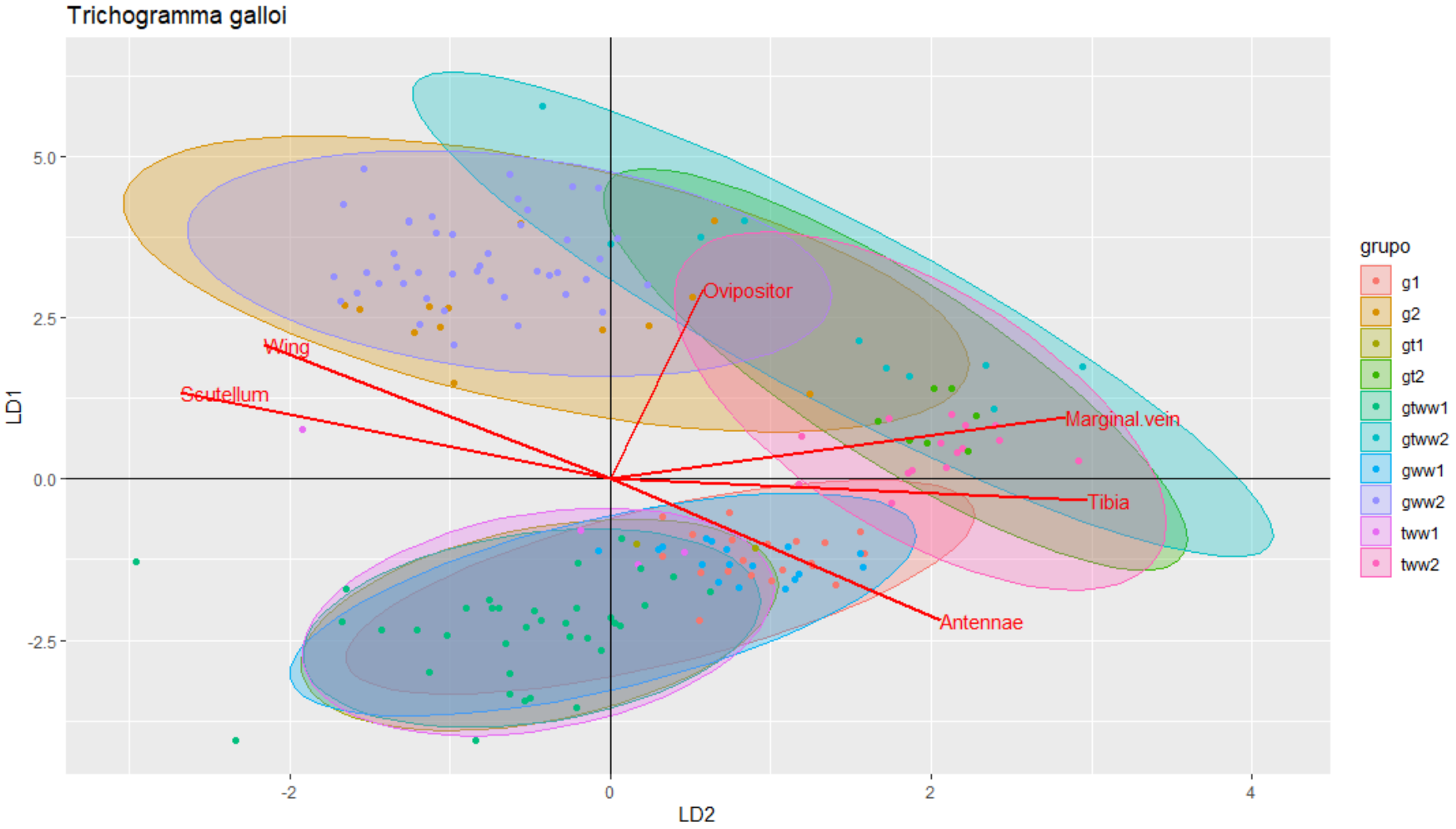
Trichogramma galloi
LD1
LD2
5.0
2.5
0.0
-2.5
-2
0
2
4
Ovipositor
Wing
Scutellum
Marginal.vein
Tibia
Antennae
grupo
g1
g2
gt1
gt2
gtww1
gtww2
gww1
gww2
tww1
tww2

Figure 1. *Trichogramma pretiosum* and *Trichogramma galloi* body parts measured. A – Antenna, B - Scutellum, C - Ovipositor, D - Tibia, E – Wing, F - Marginal vein.

Figure 2. *Trichogramma pretiosum* and *Trichogramma galloi* body parts size span decile classification, A, used to classify body parts as, B, small or, C, large.

Figure 3. Scree plot for principal component analysis of *Trichogramma pretiosum* and *Trichogramma galloi*.

Figure 4. Principal component analysis for *Trichogramma pretiosum* and *Trichogramma galloi* using the six body parts measured.

Figure 5. Linear discriminant analysis for *Trichogramma pretiosum* for groups G1, G2, T1, T2, WW1, WW2.

Figure 6. Linear discriminant analysis for *Trichogramma pretiosum* for groups GT1, GT2, GWW1, GWW2, TWW1, TWW2.

Figure 7. Linear discriminant analysis for *Trichogramma pretiosum* for groups G1, G2, GT1, GT2, GTWW1, GTWW2, GWW1, GWW2, TWW1, TWW2.

Figure 8. Linear discriminant analysis for *Trichogramma galloi* for groups G1, G2, T1, T2, WW1, WW2.

Figure 9. Linear discriminant analysis for *Trichogramma galloi* for groups GT1, GT2, GWW1, GWW2, TWW1, TWW2.

Figure 10. Linear discriminant analysis for *Trichogramma galloi* for groups G1, G2, GT1, GT2, GTWW1, GTWW2, GWW1, GWW2, TWW1, TWW2.

ARTIGO II:

LOWER TEMPERATURES PROMOTES EGG SIZE AUGMENTATION IN *Ephestia kuehniella*

Normas do periódico The Canadian Entomologist (versão preliminar)

LOWER TEMPERATURES PROMOTES EGG SIZE AUGMENTATION IN *Ephestia kuehniella* (Lepidoptera: Pyralidae)

Diego de Souza[1*], Geraldo Andrade Carvalho[2], Lucas Del Bianco Faria[3]

[1]*Graduate Program in Entomology, Department of Entomology, Federal University of Lavras, Mailbox 3037, Zip code 37200-900, Lavras, MG, Brazil, https://orcid.org/0000-0001-6759-6919*

[2]*Department of Entomology, Federal University of Lavras, Mailbox 3037, Zip code 37200-900, Lavras, MG, Brazil, https://orcid.org/0000-0002-8379-8008*

[3]*Department of Ecology and Conservation, Institute of Natural Science, Federal University of Lavras, Mailbox 3037, Zip code 37200-900, Lavras, MG, Brazil, https://orcid.org/0000-0002-7230-800X*

*Author of correspondence: Diego de Souza, desouza.diego@live.com

ABSTRACT

Egg size contribute to the quality of egg parasitoids produced for biological control. Parasitoids emerging from larger egg host are larger, more fecund, longer-lived and have the highest host location success. In this paper we evaluate *Ephestia kuehniella* egg size in different temperatures and the relationship between *E. kuehniella* egg size and parasitoid size. *Ephestia kuehniella* larvae was reared in artificial diet at 25°C, and pupae and adults were reared at 20°C, 22.5°C and 25°C. *E. kuehniella* eggs were collected, photographed and the photos used to estimate egg size as cross-sectional area. *Trichogramma pretiosum* and *Trichogramma galloi* were mounted on slides for microscopy. Slides were photographed and the photos were used to measure their ovipositor and wing, and the relationship between the biggest parasitoid and host egg size were evaluated by the proportion of large parasitoids that came from large host eggs. Mean *E. kuehniella* egg size was evaluated by linear mixed effect model. *Ephestia kuehniella* egg size increase as temperature decrease. At the same temperature, egg size slight decreases over day. We conclude that bigger eggs are produced at 20°C, and large *T. pretiosum* and *T. galloi* emerge from large host egg, but this relationship is not unanimous.

Keywords: Parasitoid size, Biological control, Quality control, Host egg.

INTRODUCTION

Insect temperature experienced during developmental and adult instars can affect egg size. Studies show that females lay larger eggs when reared or ovipositing at lower temperatures, suggesting an environment-induced egg size control (Campbell 1962; Harvey 1983; Fox and Czesak 2000). The temperature may affect the lasts instars, and ovariole development occurs in those periods (Fischer et al. 2004). Indeed, the direct effect of temperature on egg size appears to be in the pupal stage (Harvey 1983; Fischer et al. 2004). Recent studies on the effect of temperature on egg size found that long lived insects lay larger eggs at lower temperature by adjust the insect's physiology in response to oviposition temperature (Fischer et al. 2003b; Fischer et al. 2003c; Fischer et al. 2004; Steigenga et al. 2005).

Environmental factors affect egg size (Harvey 1983; Fischer et al. 2004; Steigenga et al. 2005; Amiri et al. 2020). *Apis mellifera* Linnaeus, 1758 (Hymenoptera: Apidae) queens tend to produce larger eggs in large and pollen-restricted colonies, hypothesizing that less optimal conditions induce queens to invest in larger eggs to increase survivorship of progeny (Harvey 1977; Vijendravarma et al. 2010; Amiri et al. 2020). The day an egg is laid influences its size, tending egg size to decline with the female age (Boggs 1986; Fox and Czesak 2000; Fischer et al. 2003b). *Chilo partellus* Charles Swinhoe, 1885 (Lepidoptera: Pyralidae), females lay more massive egg batches on the first day of adult life, and larger females are expected to lay more and larger eggs (Berger 1989). The number of eggs decreases with female age, but there is little information about the egg size, whereas it is known that egg weight remains constant in some cases (Berger 1989)

and decreased in the majority of studies (Harvey 1977; Jones et al. 1982; Karlsson and Wiklund 1984; Kimura and Tsubaki 1985; Boggs 1986; Wickman and Karlsson 1987; Ruohomäki et al. 1993; Oberhauser 1997). This behavior is expected to be a response to time, as time is a limiting factor for ovipositing females which tend to invest in their first progenies to increase reproductive success (Wiklund and Persson 1983; Karlsson and Wiklund 1984; Wiklund and Karlsson 1984; Karlsson 1987).

The worldwide used host egg for rearing parasitoids from the genus *Trichogramma* (Hymenoptera: Trichogrammatidae) is the flour moth *Ephestia kuehniella* Zeller, 1879 (Lepidoptera: Pyralidae) (Coelho and Parra 2013; Cherif et al. 2021; Gowda et al. 2021). In the mass rearing of *E. kuehniella*, it only feeds during larval stages, resulting in females with limited energy reserves for egg production during lifetime (Coelho and Parra 2013; Parra et al. 2014). According to the 'resource depletion' hypothesis, the best eggs are laid first due to a progressive depletion of maternal food reserves during egg production, which result in a decrease in reproductive effort with female age expressed by a decrease in the number of eggs laid per day and decrease in egg size (Campbell 1962; Wellington 1965; Torres-Vila and Rodríguez-Molina 2002). Studies on insect egg size are from old dates, but there is an agreement that arthropods exhibit substantial variation in egg size among species, among individuals within species, and sometimes even among eggs produced by a single female (Fox and Czesak 2000). *Ephestia kuehniella* egg-laying occurs over four to seven days, but most eggs are laid in the first four days (Parra et al. 2014). As *E. kuehniella* has a short lifetime, is expected the effect of temperature on egg size to be in the final stages of immature

development, permitting the young adult to show a developmental plastic response of egg size to temperature as soon it emerges (Fischer et al. 2003c).

Egg parasitoids produced for biological control are reared in factitious hosts due to rearing costs and feasibility (Coelho and Parra 2013). One quality parameter that is highly associated with overall parasitoid's quality is parasitoid size (Kazmer and Luck 1995; Bennett and Hoffmann 1998; Kölliker-Ott et al. 2003), which is dependent on two major factors, the number of parasitoids that emerges from an egg host, and the host egg size (Bai et al. 1992; Kazmer and Luck 1995; Grenier et al. 2001; Boivin and Martel 2012). Females emerging from larger hosts are larger, more fecund, longer-lived, and have the highest host location success in the field (Bai et al. 1992; Kazmer and Luck 1995; Durocher-Granger et al. 2011). Thus, the relationship between host egg size and parasitoid size seems to be an important characteristic to be evaluated.

Therefore, temperature and egg-laying day are two critical factors that affect egg size and can be managed in the *E. kuehniella* mass rearing. Moreover, host egg size influences the adult parasitoid size that emerges from it. In order to produce larger eggs to supply with better resources egg parasitoid production, this study aims to evaluate the egg size variability of *E. kuehniella* in different temperatures and egg-laying days. Also, we aim to evaluate the relationship between host egg size and parasitoid size. We hypothesize that larger eggs are produced in lower developmental temperatures and egg size diminishes over days, and the bigger the host egg, the bigger the parasitoid size.

MATERIAL AND METHODS

Ephestia kuehniella colony

Ephestia kuehniella colony was from the Department of Entomology at 'Universidade Federal de Lavras' (21.2399º S, 44.9984º W). They were reared in a climate room with controlled conditions (25±1°C, 60±5.0% RH, 12 h photophase) using a diet composed of 97% of wheat flour and 3% of Brewer's yeast (Coelho and Parra 2013). Six trays (35 cm × 29 cm × 6 cm) were prepared putting 1 kg of diet inside it and gently pressed to achieve an ideal consistency for pupation. The tray has an opening covered with *voile* tissue to permit gaseous exchange and prevent the attack of *Bracon hebetor* Say, 1836 (Hymenoptera: Braconidae), a parasitoid of *E. kuehniella* larvae. In each tray 0.3 gram of *E. kuehniella* eggs was inoculated, then closed and sealed with adhesive tape to prevent larvae escape. The trays were left inside the climate room until the pre-pupa/pupae stage, which is about 50 - 55 days after inoculation of eggs (Pakyari et al. 2019; Moghadamfar et al. 2020). Two trays were transferred to an incubator chamber regulated at 20ºC and others two at 22.5°C to compose our experimental treatment, and the others two remained at 25°C, composing our control treatment. When adults start to emerge, they were transferred to experimental PVC cages (30 cm × 30 cm), closed with metal gratings that allow oviposition and facilitate the egg's collection. Each cage contained about 100 specimens (50% female and 50% male). As just a small number of adults emerged from each tray, one cage was set up from each temperature. Adults were left to mate and the females start the oviposition in the same temperature experienced during pre-pupa/pupae period.

Egg sampling

The eggs were collected at 9 a.m. during the first four days of the ovipositing period, as it is the time when about 85% of eggs are laid (Parra et al. 2014). One hundred forty eggs were chosen randomly for measurement from each temperature and day. Since not enough individuals emerged simultaneously in each temperature treatment, the experimental and control samples were formed 5 - 7 days apart. However, within each sample, all the individuals were from the same cohort.

Egg size measurement

Eggs of *E. kuehniella* are very small in size and are nearly perfect ellipsoid (Cônsoli et al. 1999), making it difficult to measure. Therefore, each egg was photographed with a digital camera AmScope MU1003 connected to a binocular microscope OLYMPUS BX40, and the cross-sectional area (mm^2) occupied by the egg in the photo was used as an estimation of egg size (Fischer et al. 2004). The K-nearest neighbor approach was used to capture egg's area. This approach was used to split the image into foreground and background assuming that they have different colors. This is a semi-interactive approach where the user marks some pixels as "foreground" and others as "background", then is up to the kNN algorithm in the *nabor* package to classify the rest of the pixels (Mangenat and Jefferis 2017). After the classification, the number of pixels representing the egg was used to quantify the area, which was used as an estimation of egg size. All images were analyzed using R, version 4.2.2 (R Core Team 2022).

Eggs and parasitoids

Parasitoids used in the experiment were offered by Koppert Biological Systems® from Brazil. The commercial products "Pretiobug®" and "Galloibug®", composed by *T. pretiosum* and *T. galloi*, respectively, were received as pupae inside of *E. kuehniella* eggs. For each specie, 600 parasitized *E. kuehniella* eggs were photographed, and each egg was individualized in 0,5 mL Eppendorf® microtube until adult emergency. The adults were kept in the Eppendorf® microtubes with 70% alcohol until triage for body size measurement, and just females were measured.

Parasitoid size

Parasitoids were mounted using Hoyer medium in ventro/dorsal position on slides for microscopy. Slides were photographed, and the photos were used to measure the ovipositor length, measured by the most inner to the farther extreme (Fig. 1A) and the wing, measured from the beginning to the end of the MA vein (Fig. 1B). Large females were considered the ones that have the ovipositor and wing pertaining to the 8 to 10^{th} deciles of their size distribution (for more information on body size classification see Chapter 1).

Statistical analysis

Normality and presence of outliers of egg size per day and temperature were analyzed by applying Shapiro-Wilk's test and plotting Cleveland dot plot, respectively. A linear mixed effect model was fit to the data to analyze the influence of temperature on egg size and means was compared by Tukey test (5% significance). A significant temperature effect indicates a change in egg size over

temperature experienced. The influence of egg-laying day over egg size was analyzed just descriptively as our experimental design was limited and day was dependent of temperature and population. The large specimens were compared with the number of eggs classified as large. Egg was classified in the same way that females were (See "Parasitoid size" and Chapter 1), and the specimens were compared using simple rule of three. All analysis were performed using R, version 4.2.2 (R Core Team 2022).

RESULTS AND DISCUSSION

Ephestia. kuehniella pupae was exposed to lower temperature and the emerged adult females were kept at the same temperature experienced during pupae stage expecting the production of bigger eggs as the temperature decrease. Was observed that eggs from the standard rearing temperature (25°C) had the widest range and the smallest mean. Eggs from the minimal temperature (20°C) had the biggest mean, and eggs from the intermediate temperature (22.5°C) showed intermediate size (Table 1; Fig. 2), all statistically different (Table 2). Moreover, egg size diminishes over days, being eggs from the second, third and fourth days slight smaller than eggs laid on the first day (Table 1).

Those results are in line with the expectation that larger eggs are laid in lower temperatures and agree with the very best studied insect about egg size plasticity *Bicyclus anynana* Butler, 1879 (Lepidoptera: Nymphalidae) where for the first time a clear plastic response to rearing and oviposition temperature was observed. As egg maturation in butterflies starts in the pupal or early adult phase, the lower temperature in the pupal phase was the major contributor to the egg plastic

response observed. Despite this temperature-size relationship being largely reported in the literature, the underlying mechanism and its adaptative significance are unknown (Fischer et al. 2004). A possible explanation could be the acclimatation expected for insects that have a longer lifetime, which permits the insect to "sense" the weather and adjust their egg size to the temperature experienced during oviposition (Fischer et al. 2003a).

We highlight that the larval temperature (25ºC) was the same in all treatments, but pupae and early adult temperature were different. We chose to maintain adults at the same temperature as pupae because it's known that exist an acclimatation mechanism which could "reverse" the influence of low rearing temperature on egg size (Fischer et al. 2003b; Fischer et al. 2003c; Fischer et al. 2004). Fischer et al. (2003c) observed that females reared in high temperature (27°C) oviposited smaller eggs than females reared in low temperature (20°C), but after 10 days ovipositioning in lower temperature (20°C) females started to lay bigger eggs. The reverse was also true, females reared in low temperatures (20°C) oviposited bigger eggs than females reared in high temperatures (27°C) and after 10 days ovipositioning in high temperature (27°C) females started to lay smaller eggs.

For insects that have a short lifetime, acclimatation is difficult to occur and measure, thus the effect of temperature is more evident for younger adults. Despite the short egg laying period of 4 days seems unlikely to cause an "acclimatation effect" in egg size, was observed that eggs became slight smaller as the days pass over. It could be the effect of the decrease of resources available for egg production,

200 or a resource depletion caused by advanced female age (Fox and Czesak 2000;
201 Steigenga et al. 2005). As *E. kuehniella* only feed during larval stages, the reserves
202 acquired are consumed for egg production and other life activities, remaining fewer
203 reserves through time.

204 One point to highlight and evaluate in futures experiments is if lower
205 temperature during pupal and adult phase could interfere with biological parameters
206 important for egg production other than egg size, e.g., longevity, fecundity,
207 emergency and, sex ratio. This paper aim to evaluate egg size, but there is evidence
208 that females kept at lower temperatures live longer, which could extend the lifetime
209 for egg production (Coelho and Parra 2013). There are evidence indicating a trade-
210 off between egg size and egg number (Fischer and Fiedler 2001), and for *E.*
211 *kuehniella* reared as larvae and pupae in lower temperatures, more and heavier eggs
212 are laid (Coelho and Parra 2013).

213 The last question addressed in this paper was the relationship between
214 parasitoid size and the egg size it emerges. The two major factors determining
215 parasitoid size are the number of parasitoids that emerges from an egg host, and the
216 host egg size (Bai et al. 1992; Kazmer and Luck 1995; Boivin and Martel 2012). In
217 our analysis, just eggs that reared one parasitoid were used. The key point in
218 enhancing the host egg size in the natural enemies' mass rearing is because large
219 eggs could generate large parasitoids, which is believed to be more efficient in the
220 pest control. *Trichogramma* spp. females that emerge from large eggs are the
221 biggest, but this relationship is not unanimous. We observed that 32 large *T.*
222 *pretiosum* females out of 47 came from large eggs, which represents 68% of the

population, and 29 large *T. galloi* females out of 53, which represents 54,70%. Considering all 484 eggs analyzed, 141 eggs were classified as large, which correspond to 29,13%. From those 141 large eggs, 43,26% originated large females.

Our expectation that large specimens came from large eggs were partially satisfied. First, the size of the body parts measured does not have high correlations with egg size (data not shown). Second, egg size does not guarantee large specimens as just 43% of large eggs reared large specimens. The fact that eggs were received with *T. pretiosum* and *T. galloi* in the pupae stage could explain the low correlation between egg size and parasitoid size. As the larvae consume the egg yolk, the egg could diminish and warp, which could influence the egg size measurement. Studies on the relationship between egg size and parasitoid size can rely on different host species to generate specimens of different size (Bai et al. 1992; Kazmer and Luck 1995). In our study, small and large specimens emerged from the same host specie *E. kuehniella*. Thus, we believe that the parasitoid size variability does not rely just on the egg size and more studies need to be done in order to understand the factors involved in parasitoid size.

In summary, our results show that larger eggs are laid in lower temperatures and there are a slightly decrease in size over days. Moreover, large parasitoid does emerge from large host egg, but this relationship is not unanimous. Based on the mass rearing insect production biograph, our results give important light to enhance the quality of *E. kuehniella* eggs offered to egg parasitoids, which could enhance the quality of parasitoids reared on those eggs. As eggs of most parasitoid wasps reared for biological control have no yolk, the host egg must provide all the essential

nutrients to the parasitoid development. Different hosts eggs are nutritional and sized distinct, which could influence the parasitoid performance (Cascone et al. 2015). Bigger eggs are expected to provide at least more nutritional content than smaller ones, which in turn lead to the production of parasitoids with higher quality (Farahani et al. 2016; Moghaddassi et al. 2019; Alcalá et al. 2021).

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REFERENCES

Acevedo-Alcalá, A., Rodríguez-Leyva, E. and Lomeli-Flores, J.R., 2021. Impact of Host Size and Honey Availability on *Trichogramma atopovirilia* (Hymenoptera: Trichogrammatidae) Longevity. Journal of Entomological Science, 56(3), p.458-461. https://doi.org/10.18474/JES20-73

Amiri, E., Le, K., Melendez, C.V., Strand, M.K., Tarpy, D.R. and Rueppell, O., 2020. Egg-size plasticity in *Apis mellifera*: honey bee queens alter egg size in response to both genetic and environmental factors. Journal of Evolutionary Biology, 33(4), p.534-543. https://doi.org/10.1111/jeb.13589

Bai, B., Luck, R.F., Forster, L., Stephens, B. and Janssen, J.M., 1992. The effect of host size on quality attributes of the egg parasitoid, *Trichogramma pretiosum*. Entomologia Experimentalis et Applicata, 64(1), p.37-48. https://doi.org/10.1111/j.1570-7458.1992.tb01592.x

Berger, A., 1989. Egg weight, batch size and fecundity of the spotted stalk borer, *Chilo partellus* in relation to weight of females and time of oviposition. Entomologia Experimentalis et Applicata, 50(3), p.199-207. https://doi.org/10.1111/j.1570-7458.1989.tb01193.x

Bennett, D.M. and Hoffmann, A.A., 1998. Effects of size and fluctuating asymmetry on field fitness of the parasitoid *Trichogramma carverae* (Hymenoptera: Trichogrammatidae). Journal of Animal Ecology, p.580-591. https://www.jstor.org/stable/2647279

BOGGS, C.L., 1986. Reproductive strategies of female butterflies: variation in and constraints on fecundity. Ecological Entomology, 11(1), p.7-15. https://doi.org/10.1111/j.1365-2311.1986.tb00274.x

Boivin, G. and Martel, V., 2012. Size-induced reproductive constraints in an egg parasitoid. Journal of Insect Physiology, 58(12), p.1694-1700. https://doi.org/10.1016/j.jinsphys.2012.10.014

Campbell, I.M., 1962. Reproductive capacity in the genus *Choristoneura* Led. (Lepidoptera: Tortricidae). I. Quantitative inheritance and genes as controllers of rates. Canadian Journal of Genetics and Cytology, 4(3), p.272-288. https://doi.org/10.1139/g62-036

Cascone, P., Carpenito, S., Slotsbo, S., Iodice, L., Sørensen, J.G., Holmstrup, M. and Guerrieri, E., 2015. Improving the efficiency of *Trichogramma achaeae* to control *Tuta absoluta*. BioControl, 60(6), p.761-771. https://doi.org/10.1007/s10526-015-9684-1

Cherif, A., Ramzi Mansour, R., and Grissa-Lebdi, K. 2021. The egg parasitoids *Trichogramma*: from laboratory mass rearing to biological control of lepidopteran pests. Biocontrol Science and Technology, 31:7, 661-693. https://doi.org/10.1080/09583157.2020.1871469

Cônsoli, F.L., Kitajima, E.W. and Parra, J.R.P., 1999. Ultrastructure of the natural and factitious host eggs of *Trichogramma galloi* Zucchi and *Trichogramma pretiosum* Riley (Hymenoptera: Trichogrammatidae). International Journal of Insect Morphology and Embryology, 28(3), p.211-231. https://doi.org/10.1016/S0020-7322(99)00026-4

Coelho Jr, A. and Parra, J.R.P., 2013. The effect of rearing in different temperature regimes on the reproduction of *Anagasta kuehniella* (Lepidoptera: Pyralidae). Environmental Entomology, 42(4), p.799-804. https://doi.org/10.1603/EN12106

Durocher-Granger, L., Martel, V. and Boivin, G., 2011. Gamete number and size correlate with adult size in the egg parasitoid *Trichogramma euproctidis*. Entomologia Experimentalis et Applicata, 140(3), p.262-268. https://doi.org/10.1111/j.1570-7458.2011.01158.x

Farahani, H.K., Ashouri, A., Zibaee, A., Abroon, P. and Alford, L., 2016. The effect of host nutritional quality on multiple components of *Trichogramma brassicae* fitness. Bulletin of Entomological Research, 106(5), p.633-641. https://doi.org/10.1017/S000748531600033X

Fischer, K. and Fiedler, K., 2001. Egg weight variation in the butterfly *Lycaena hippothoe*: more small or fewer large eggs? Population Ecology, 43(1), p.105-109. https://doi.org/10.1007/PL00012009

Fischer, K., Bot, A.N., Brakefield, P.M. and Zwaan, B.J., 2003a. Fitness consequences of temperature-mediated egg size plasticity in a butterfly. Functional Ecology, p.803-810. https://www.jstor.org/stable/3599252

Fischer, K., Brakefield, P.M. and Zwaan, B.J., 2003b. Plasticity in butterfly egg size: why larger offspring at lower temperatures? Ecology, 84(12), p.3138-3147. https://doi.org/10.1890/02-0733

Fischer, K., Eenhoorn, E., Bot, A.N., Brakefield, P.M. and Zwaan, B.J., 2003c. Cooler butterflies lay larger eggs: developmental plasticity versus acclimation. Proceedings of the Royal Society of London. Series B: Biological Sciences, 270(1528), p.2051-2056. https://doi.org/10.1098/rspb.2003.2470

Fischer, K., Bot, A.N.M., Zwaan, B.J. and Brakefield, P.M., 2004. Genetic and environmental sources of egg size variation in the butterfly Bicyclus anynana. Heredity, 92(3), p.163-169. https://doi.org/10.1038/sj.hdy.6800382

Fox, C.W. and Czesak, M.E., 2000. Evolutionary ecology of progeny size in arthropods. Annual Review of Entomology, 45(1), p.341-369. https://doi.org/10.1146/annurev.ento.45.1.341

Gowda B.G., Pandi G, G.P., Ullah, F., Patil, N.B., Sahu, M., Adak, T., Pokhare, S., Yadav, M.K., Mahendiran, A., Mittapelly, P., Desneux, N. and Rath, P.C., 2021. Performance of Trichogramma japonicum under field conditions as a function of the factitious host species used for mass rearing. Plos one, 16(8), p.e0256246. https://doi.org/10.1371/journal.pone.0256246

Grenier, S., Basso, C. and Pintureau, B., 2001. Effects of the host species and the number of parasitoids per host on the size of some Trichogramma species (Hymenoptera: Trichogrammatidae). Biocontrol Science and Technology, 11(1), p.21-26. https://doi.org/10.1080/09583150020029709

Harvey, G.T., 1977. Mean weight and rearing performance of successive egg clusters of eastern spruce budworm (Lepidoptera: Tortricidae). The Canadian Entomologist, 109(4), p.487-496. https://doi.org/10.4039/Ent109487-4

Harvey, G.T., 1983. Environmental and genetic effects on mean egg weight in spruce budworm (Lepidoptera: Tortricidae). The Canadian Entomologist, 115(9), p.1109-1117. https://doi.org/10.4039/Ent1151109-9

Jones, R.E., Hart, J.R. and Bull, G.D., 1982. Temperature, size and egg production in the cabbage butterfly, *Pieris rapae* L. Australian Journal of Zoology, 30(2), p.223-231. https://doi.org/10.1071/ZO9820223

Karlsson, B. and Wiklund, C., 1984. Egg weight variation and lack of correlation between egg weight and offspring fitness in the wall brown butterfly *Lasiommata megera*. Oikos, p.376-385. https://doi.org/10.2307/3544156

Karlsson, B., 1987. Variation in egg weight, oviposition rate and reproductive reserves with female age in a natural population of the speckled wood butterfly, *Pararge aegeria*. Ecological Entomology, 12(4), p.473-476. https://doi.org/10.1111/j.1365-2311.1987.tb01029.x

385 Kazmer, D.J. and Luck, R.F., 1995. Field tests of the size-fitness hypothesis in the
386 egg parasitoid *Trichogramma pretiosum*. Ecology, 76(2), p.412-425.
387 https://doi.org/10.2307/1941200

388

389 Kölliker-Ott, U.M., Blows, M.W. and Hoffmann, A.A., 2003. Are wing size, wing
390 shape and asymmetry related to field fitness of *Trichogramma* egg parasitoids?.
391 Oikos, 100(3), p.563-573. https://doi.org/10.1034/j.1600-0706.2003.12063.x

392

393 Kimura, K. and Tsubaki, Y., 1985. Egg weight variation associated with female
394 age in *Pieris rapae* crucivora Boisduval (Lepidoptera: Pieridae). Applied
395 Entomology and Zoology, 20(4), p.500-501. https://doi.org/10.1303/aez.20.500

396

397 Mangenat, S. and Jefferis, G. (2017) nabor: Wraps 'libnabo', a Fast K Nearest
398 Neighbour Library for Low Dimensions. R Package v 0.5.0. Available at:
399 https://CRAN.R-project.org/package=nabor (accessed on 01 November 2022)

400

401 Moghadamfar, Z., Pakyari, H. and Amir-Maafi, M., 2020. Age-stage, two-sex life
402 table of *Ephestia kuheniella* (Lep: Pyrallidae) at different constant temperatures.
403 Crop Protection, 137, 105200. https://doi.org/10.1016/j.cropro.2020.105200

404

405 Moghaddassi, Y., Ashouri, A., Bandani, A.R., Leppla, N.C. and Shirk, P.D., 2019.
406 Effect of *Ephestia kuehniella* (Lepidoptera: Pyralidae) larval diet on egg quality
407 and parasitism by *Trichogramma brassicae* (Hymenoptera: Trichogrammatidae).
408 Journal of Insect Science, 19(4), 10. https://doi.org/10.1093/jisesa/iez076

409

410 Oberhauser, K.S., 1997. Fecundity, lifespan and egg mass in butterflies: effects of
411 male-derived nutrients and female size. Functional Ecology, 11(2), p.166-175.
412 https://doi.org/10.1046/j.1365-2435.1997.00074.x

413

414 Parra, J. R. P., Coelho Jr, A., Geremias, L. D., Bertin, A., and Ramos, C. J., 2014.
415 Criação de *Anagasta kuehniella*, em pequena escala, para produção de
416 *Trichogramma*. Occasio, 32 p.:il.

417

418 Pakyari, H., Amir-Maafi, M., Moghadamfar, Z. and Zalucki, M., 2019. Estimating
419 development and temperature thresholds of *Ephestia kuehniella*: toward
420 improving a mass production system. Bulletin of Entomological Research, 109(4),
421 p.435-442. https://doi.org/10.1017/S0007485318000718

R Core Team. 2022. R: a language and environment for statistical computing. Available from https://www.r-project.org/ [accessed 1 November 2022]

Ruohomäki, K., Hanhimäki, S. and Haukioja, E., 1993. Effects of egg size, laying order and larval density on performance of *Epirrita autumnata* (Lep., Geometridae). Oikos, p.61-66. https://doi.org/10.2307/3545309

Steigenga, M.J., Zwaan, B.J., Brakefield, P.M. and Fischer, K., 2005. The evolutionary genetics of egg size plasticity in a butterfly. Journal of Evolutionary Biology, 18(2), p.281-289. https://doi.org/10.1111/j.1420-9101.2004.00855.x

Torres-Vila, L.M. and Rodríguez-Molina, M.C., 2002. Egg size variation and its relationship with larval performance in the Lepidoptera: the case of the European grapevine moth *Lobesia botrana*. Oikos, 99(2), p.272-283. https://doi.org/10.1034/j.1600-0706.2002.990207.x

Vijendravarma, R.K., Narasimha, S. and Kawecki, T.J., 2010. Effects of parental larval diet on egg size and offspring traits in *Drosophila*. Biology letters, 6(2), p.238-241. https://doi.org/10.1098/rsbl.2009.0754

Wellington, W.G., 1965. Some maternal influences on progeny quality in the western tent caterpillar, *Malacosoma pluviale* (Dyar). The Canadian Entomologist, 97(1), p.1-14. https://doi.org/10.4039/Ent971-1

Wiklund, C. and Persson, A., 1983. Fecundity, and the relation of egg weight variation to offspring fitness in the speckled wood butterfly *Pararge aegeria*, or why don't butterfly females lay more eggs? Oikos, p.53-63. https://doi.org/10.2307/3544198

Wiklund, C. and Karlsson, B., 1984. Egg size variation in satyrid butterflies: adaptive vs historical," Bauplan", and mechanistic explanations. Oikos, p.391-400. https://doi.org/10.2307/3544158

Wickman, P.O. and Karlsson, B., 1987. Changes in egg color, egg weight and oviposition rate with the number of eggs laid by wild females of the small heath

458 butterfly, *Coenonympha pamphilus*. Ecological Entomology, 12(1), p.109-114.
459 https://doi.org/10.1111/j.1365-2311.1987.tb00989.x

460 Table 1 Maximum, mean, and minimum egg size (mm^2), standard deviation,
461 Shapiro test and *P* value for *Ephestia kuehniella* eggs laid on the first, second,
462 third and fourth day at temperatures 25°C, 22.5°C and 20°C.

Temperature	Day	Max	Mean	Min	SD	Shapiro-Wilk test	*P*
25°C	1	0.1749	0.1410	0.1222	0.00813581	0.95344	0.000115
	2	0.1642	0.1411	0.1127	0.008094293	0.98252	0.07074
	3	0.1553	0.1384	0.1125	0.007598434	0.99017	0.4322
	4	0.1517	0.1340	0.1155	0.0066569	0.99255	0.6762
22.5°C	1	0.1727	0.1422	0.1256	0.006846313	0.96026	0.0004409
	2	0.1583	0.1408	0.1256	0.006558431	0.99208	0.6252
	3	0.1640	0.1384	0.1140	0.007461174	0.98157	0.05592
	4	0.1558	0.1374	0.1210	0.006952141	0.99098	0.5099
20°C	1	0.1724	0.1447	0.1262	0.007877149	0.96285	0.0007536
	2	0.1698	0.1453	0.1216	0.007980606	0.99236	0.6554
	3	0.1632	0.1420	0.1232	0.007282024	0.99621	0.9763
	4	0.1590	0.1394	0.1203	0.007377341	0.99379	0.8067

463

464 Table 2. Linear mixed effects model for mean *Ephestia kuehniella* egg size in
465 three temperatures.

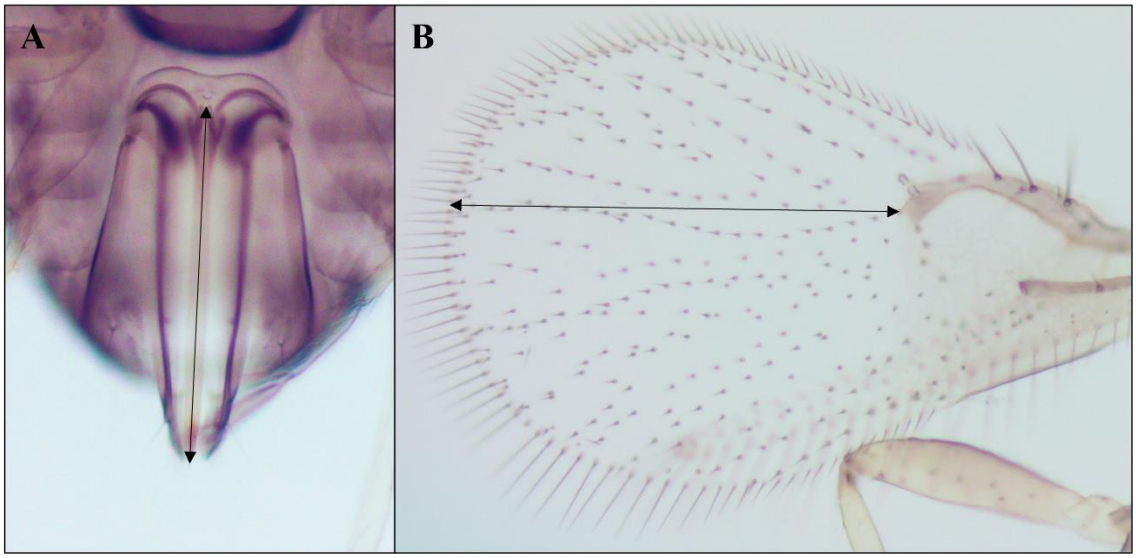
Temperature	Estimate	Std. Error	df	t value	Pr(>|t|)
20°C	1.429e-01	1.427e-03	3.727e+00	100.113	< 0.05
22.5°C	-3.185e-03	4.425e-04	1.671e+03	-7.198	< 0.05
25°C	-4.242e-03	4.425e-04	1.671e+03	-9.587	< 0.05

466

Figure 1. *Trichogramma pretiosum* and *Trichogramma galloi* body parts measured. A – Ovipositor, B - Wing.

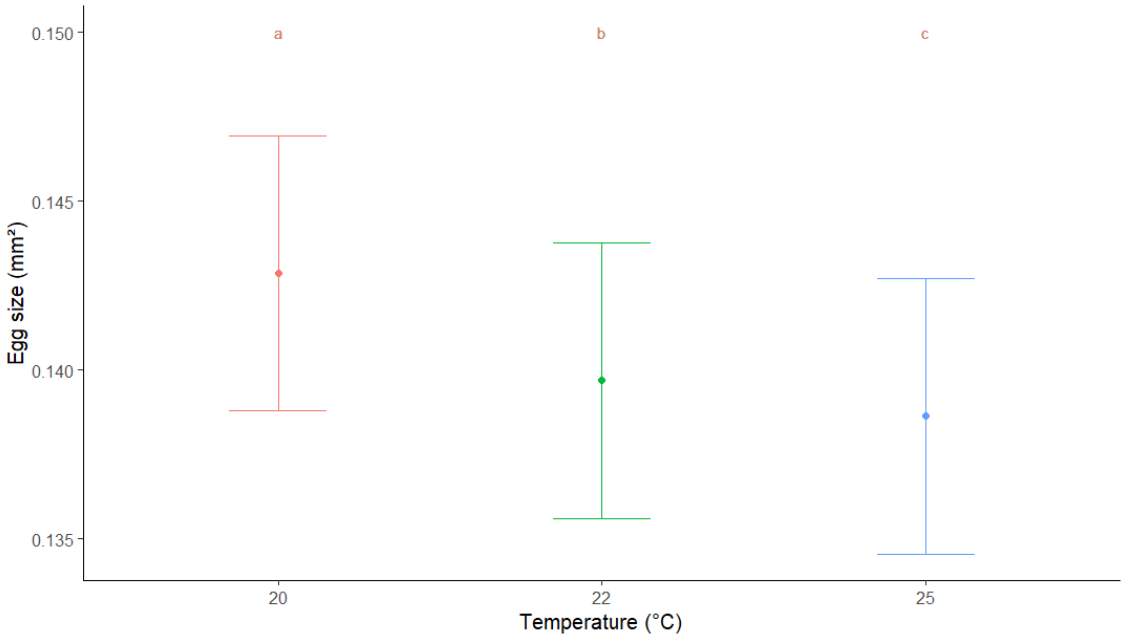
Figure 2. *Ephestia kuehniella* egg size (mm^2) in three temperatures.

470 Figure 1



471

472 Figure 2



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