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LUCIANA ORDUNHA ARARIPE

"VARIAÇÃO GENÉTICA E MORFOLÓGICA EM POPULAÇÕES DE *ZAPRIONUS INDIANUS* (DIPTERA: DROSOPHILIDAE)"

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Orientador: Prof. Dr. Louis Bernard Klaczko

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Banca Examinadora

Prof. Dr. Louis Bernard Klaczko (Orientador) _____
Assinatura

Profa. Dra. Vera Nisaka Solferini _____
Assinatura

Prof. Dr. Sérgio Furtado dos Reis _____
Assinatura

Profa. Dra. Rosana Tidon _____
Assinatura

Prof. Dr. Sérgio Russo Matioli _____
Assinatura

Prof. Dr. Gabriel Marroig _____
Assinatura

Profa. Dra. Ana Maria Lima de Azeredo-Espin _____
Assinatura

E é por ela ser assim tão delicada

Que eu trato sempre dela muito bem.

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RESUMO

A espécie *Zaprionus indianus* expandiu sua distribuição geográfica recentemente, a partir da invasão do continente sul-americano. O primeiro registro data de apenas seis anos, e acredita-se que a origem seja sul-africana. Hoje, indivíduos dessa espécie são encontrados numa amplitude latitudinal de 35^{o}, do Uruguai a Belém (Brasil). A chegada de *Z. indianus* no Brasil apresenta uma oportunidade rara de se estudar um evento de invasão desde seu início. O estudo de características fisiológicas, morfológicas e genéticas de populações brasileiras pode ajudar a construir hipóteses para o sucesso de *Z. indianus* nas etapas de invasão, além de indicar o potencial para evolução e diferenciação fenotípica. Um aspecto fisiológico estudado nesta tese foi a condição reprodutiva em machos que foram submetidos a extremos de temperatura ao longo de seu desenvolvimento. O estresse térmico causa esterilidade nos machos e a investigação dos limites (superior e inferior) da curva de fertilidade relativa à temperatura, complementa os dados de viabilidade na caracterização do potencial de ocupação de áreas novas por espécies cosmopolitas. Quanto à morfologia, um aspecto estudado foi o dimorfismo sexual no número de cerdas abdominais em uma população africana. Dimorfismo sexual para este caráter foi observado em várias espécies em estudos anteriores, embora padrões diferentes tenham sido encontrados. Nesta tese o padrão observado em *Z. indianus* foi comparado com o de outra espécie cosmopolita, *Drosophila melanogaster.* Um terceiro ponto abordado nesta tese foi a investigação de 10 populações brasileiras quanto à variação fenotípica (tamanho e forma da asa). Além das diferenças entre populações, uma abordagem interessante é o quanto de variação está presente dentro de cada população. Ainda, é importante saber o quanto dessa variação é herdável para estimarmos qual a capacidade evolutiva da população. O estudo revelou a ocorrência de alta variabilidade fenotípica dentro e entre populações, além de variação genética aditiva suficiente para promover a evolução de caracteres relacionados à forma da asa.

ABSTRACT

The species *Zaprionus indianus* has recently expanded its geographical distribution with the invasion of the South American continent. The first record dates of only six years, and the origin is probably the South Africa. Nowadays, individuals of this species can be found in a latitudinal range of 35°, from Uruguai to Belém (Brazil). The arrival of *Z. indianus* in Brazil presents a rare opportunity of studying an event of invasion from its beginning. The study of physiological, morphological and genetic traits in Brazilian populations may contribute to generate hypothesis for the success of *Z. indianus* in the stages of invasion. Also, it may indicate the potential for evolution and phenotypic differentiation. One physiological trait studied in the current thesis was the relationship of fertility and the temperature of development. Extreme temperatures cause sterility in males and the study of the limits (upper and lower) of the fertility curve complements the data on viability for the characterization of the potential of cosmopolitan species to occupy new areas. One morphological character studied was the sexual dimorphism for the number of abdominal bristles in one African population. A significant sexual dimorphism for this trait was observed in several species in previous studies, though different patterns have been found. In the current work the pattern observed in *Z. indianus* was compared to the pattern for another cosmopolitan species, *Drosophila melanogaster*. The third approach of this thesis was the study of the phenotypic variation (size and shape of the wing) present in 10 Brazilian populations. Besides the differences among populations, an interesting approach is the amount of variation present within each population. Also, it is important to know the proportion of this variation that is heritable, which allows an estimate of the evolutionary potential of the population. This study revealed the occurrence of high phenotypic variability within and between populations. In addition, enough additive genetic variation exists to promote the evolution of traits related to the shape of the wing.

INTRODUÇÃO

O gênero *Zaprionus* pertence à família Drosophilidae e é nativo do continente africano. Atualmente 52 espécies estão descritas (Chassagnard & Tsacas, 1993) e distribuem-se também nas regiões Australiana, Oriental e Paleártica (Tsacas et al., 1981). Grande interesse tem sido demonstrado no estudo da espécie *Zaprionus indianus,* principalmente pela recente expansão da sua área de distribuição com a invasão do continente sul-americano, sendo considerada hoje como uma espécie semi-cosmopolita. O primeiro registro dessa espécie no Brasil ocorreu há seis anos (Vilela, 1999) no Estado de São Paulo, onde foi considerada uma praga para plantações de figos por causar a perda de 50% da produção naquele ano (Tidon et al., 2003). Nos anos seguintes *Z. indianus* expandiu sua distribuição com sucesso, sendo encontrada hoje numa amplitude latitudinal de 35^{o}, do Uruguai a Belém (Brasil).

Estima-se que a espécie *Z. indianus* já havia invadido o subcontinente indiano há alguns séculos. Um estudo recente (Karan et al., 2000) mostra que diferenças genéticas e morfológicas entre populações dessa espécie na Índia seguem um gradiente latitudinal, o que fornece uma evidência de seleção natural e adaptação local. Além disso, indica que as populações indianas têm potencial evolutivo para responder a condições externas e diferenciar-se de populações em outras localidades de distribuição da espécie.

A questão de invasões biológicas é de interesse crescente para o campo de genética evolutiva. Vermeij (1996) classificou o processo de invasão em três etapas: chegada, estabelecimento e integração. Em muitos casos de invasões biológicas a primeira etapa tem sido facilitada pelo homem, direta ou indiretamente, através do transporte de passageiros e mercadorias. As últimas duas etapas dependem de características intrínsecas da espécie invasora e assumem que esta esteja respondendo a um regime seletivo e, mais ainda, esteja se tornando

parte desse regime por sua interação com outras espécies da comunidade. A partir daí, algumas questões acerca da invasão de *Z. indianus* no Brasil tornam-se interessantes: 1) como e em quantos eventos se deu a chegada dessa espécie no Brasil? 2) quais as características biológicas que contribuem para o sucesso de sua expansão geográfica? 3) qual o efeito da colonização sobre populações de espécies nativas? 4) qual o nível de diferenciação genética e fenotípica entre populações brasileiras? 5) qual o potencial evolutivo das populações invasoras?

As invasões biológicas são em geral acidentais e, portanto, é muito difícil obter-se uma informação segura da data, origem e condições em que indivíduos de *Z. indianus* chegaram ao Brasil. Como esta é uma espécie muito conspícua e nas primeiras coletas apareceu em freqüências muito baixas (Vilela, 1999; Tidon et al., 2003), acredita-se que tenha sido detectada pouco tempo após a invasão. A hipótese mais aceita para a introdução dessa espécie no continente sul-americano é a de que adultos ou larvas estariam presentes em alimentos oferecidos a passageiros de vôos vindos da África ou Ásia. Apesar de estar restrita aos climas tropical e subtropical quentes (Karan et al., 1999; Tidon et al., 2003; Araripe et al., 2004), *Z. indianus* mostra características generalistas e é capaz de adaptar-se a diferentes condições ambientais (Parkash & Yadav, 1993). Tal versatilidade ecológica pode ter sido a razão do sucesso na fase de estabelecimento.

O número de indivíduos presentes no evento de invasão também pode contribuir para a capacidade de ocupação e expansão da espécie no novo território. Por efeitos de amostragem, poucos indivíduos dão origem a populações com baixa variabilidade genética, no sentido de frequências alélicas extremas em diversos loci, o que pode diminuir a capacidade de adaptação a condições diversas. Por isso, como resultado de uma introdução única e acidental, espera-se encontrar baixa variabilidade genética resultante de um 'efeito gargalo'. Por outro lado, a presença de variação significativa sugeriria a chegada de muitos indivíduos ou a ocorrência de

eventos múltiplos de invasão. Apesar de tal dicotomia ser bastante aceita, alguns autores mostraram que a perda de variabilidade genética não é consequência direta de um gargalo populacional (Carson, 1990; Whitlock et al., 1993; Cheverud & Routman, 1995; 1996). Ao contrário, uma redução no tamanho populacional seguida de deriva leva as frequências alélicas a valores extremos, o que estabelece uma condição adequada para o aumento da variância genética aditiva quando os genes envolvidos mostram alguma interação entre si (epistase) – (Carson, 1990; Cheverud & Routman, 1996).

A chegada de *Z. indianus* no Brasil (Vilela, 1999; Goñi et al., 2001; Tidon et al., 2003) apresenta uma oportunidade rara de se estudar um evento de invasão desde seu início. O estudo de características fisiológicas, morfológicas e genéticas de populações brasileiras pode ajudar a construir hipóteses para o sucesso de *Z. indianus* nas etapas de invasão, além de indicar o potencial para evolução e diferenciação fenotípica.

Tolerância térmica

Uma característica importante para explicar a distribuição e abundância de animais, principalmente ectotérmicos, é a tolerância térmica. Em geral, espécies de ambientes tropicais apresentam uma sensibilidade alta ao frio e espécies de ambientes frios são sensíveis ao calor. A adaptação térmica é tradicionalmente estudada através da resposta de caracteres relacionados ao valor adaptativo, como viabilidade, fecundidade e taxa de desenvolvimento, a condições de temperaturas extremas (Hoffmann et al., 2003). Recentemente novos critérios foram sugeridos, como o tempo decorrido até a recuperação após coma induzido por frio (David et al., 1998; Gibert et al., 2001; Hoffmann et al., 2002). Esta medida fornece uma indicação direta da tolerância de indivíduos de uma determinada espécie a condições extremas de frio.

Outro critério é a investigação dos extremos de temperatura, superior e inferior, que causam a esterilidade em machos de uma determinada espécie (Chakir et al., 2002). Somado à curva de variação da fertilidade segundo a temperatura, o tempo que um macho estéril leva para recuperar a fertilidade após retornar à temperatura média (David et al., 1971; Cohet, 1973) também pode ser usado para descrever e comparar a tolerância térmica em diferentes espécies. Chakir et al. (2002) usaram esses parâmetros para comparar duas espécies cosmopolitas (*D. melanogaster* e *D. simulans*) que mostram sobreposição parcial de suas distribuições geográficas. Os extremos de temperatura causando esterilidade completa foram diferentes nas duas espécies, com *D. simulans* sendo mais tolerante ao frio e mais sensível ao calor que *D. melanogaster.*

A investigação dos limites de esterilidade em *Z. indianus* é interessante do ponto de vista ecológico e adaptativo. A amplitude térmica para viabilidade nessa espécie é de 12-32^{o}C (Karan et al., 1999), semelhante à encontrada para *D. melanogaster* (Pétavy et al., 2001). Como a esterilidade em machos também é um fator limitante para a permanência de uma população em determinado ambiente, seu estudo complementa os dados de viabilidade na caracterização do potencial de ocupação de áreas novas por espécies cosmopolitas. Com isso, o primeiro capítulo desta tese trata de um estudo semelhante ao conduzido por Chakir et al. (2002), que foi publicado em fevereiro de 2004, na revista *Journal of Thermal Biology* (Araripe et al., 2004). Os dados apresentados aqui também estão fazendo parte de uma revisão sobre o tema, que está no prelo da revista *Journal of Evolutionary Biology* (David et al., 2005).

Caracteres morfológicos

A investigação de caracteres morfológicos também contribui para a caracterização de uma espécie invasora quanto a sua capacidade de adaptação, além de ser importante na diferenciação de populações. Dentre diversos caracteres (comprimento da asa e do tórax, número de cerdas

esternopleurais e abdominais e número de ovaríolos nas fêmeas) estudados em uma população africana de *Z. indianus* proveniente da Cidade do Cabo (África do Sul), a quantificação do número de cerdas abdominais nos dois sexos sugeriu a ocorrência de fenômenos interessantes. Por isso, no segundo capítulo desta tese concentrei-me num estudo mais detalhado desse caráter.

Em adultos de *D. melanogaster,* o número de cerdas abdominais tem sido considerado como um caráter ideal para estudos de genética quantitativa (Falconer e Mackay, 1996). Algumas razões para isso são a existência satisfatória de informações sobre o desenvolvimento (Mackay, 2001; Brakefield, 2003) e sobre mutações principais afetando esse caráter (Lindsley and Zimm, 1992). Além disso, vários *loci* de caracteres quantitativos (QTL) foram identificados em populações naturais (True et al., 1997; Long et al., 1998, 2000; Nuzhdin et al., 1999; Kopp et al., 2000; Dilda & Mackay, 2002), o que facilita o estudo das bases genéticas da diferenciação desse caráter.

O desenvolvimento das cerdas começa com a expressão de genes do complexo *achaete-scute* (ASC) num agrupamento de células localizadas na epiderme. Inicialmente, todas as células expressam os complexos gênicos *Delta* e ASC. Essa expressão é gradualmente restrita a apenas uma das células do agrupamento através de um processo de inibição lateral, promovido pela via de sinalização *Notch,* que é caracterizado pela ativação de fatores de transcrição que regulam *Notch* positivamente e inibem *Delta* e ASC. A cerda é então produzida depois de divisões subseqüentes da célula que não foi inibida (Brakefield et al., 2003). Apesar da via de desenvolvimento das cerdas estar descrita aqui de maneira simples, é possível perceber que existem muitas maneiras pelas quais uma alteração poderia ocorrer levando a uma mudança fenotípica no padrão de cerdas.

A questão de que mudanças genéticas são responsáveis pela variação fenotípica observada dentro e entre espécies tem sido levantada por muitos biólogos evolutivos. Além disso, muito

interesse tem sido demonstrado no controle genético da diferenciação do número de cerdas abdominais entre sexos (Kopp et al., 2003). Apesar de serem considerados caracteres modulares, os segmentos abdominais são fortemente afetados pelo sexo do indivíduo. Fêmeas apresentam 7 segmentos, com tergitos (placas dorsais) visíveis em todos eles, e esternitos (placas ventrais) com cerdas a partir de A2 (2º segmento). Por outro lado, machos só têm 6 tergitos visíveis (A1 ao A6), sendo os dois últimos completamente escuros em *D. melanogaster*; e 6 esternitos, sendo que o último não contém cerdas. O padrão de pigmentação dos segmentos abdominais foi bem estudado em *D. melanogaster* (David et al., 1990; Gibert, 1998b, Kopp et al., 2003) e mostra grande plasticidade de acordo com a temperatura de desenvolvimento. Resultados dessas pesquisas sugerem que, apesar de genes homeóticos serem responsáveis pela diferenciação dos segmentos abdominais, um gradiente morfogenético deve estar presente, levando a interações bioquímicas complexas e, conseqüentemente, a uma resposta fenotípica diferenciada para cada tergito.

Ao contrário dos tergitos, a variação entre esternitos tem sido pouco estudada. O número de cerdas presentes nos esternitos foi analisado para algumas espécies, embora somente um ou dois segmentos subseqüentes tenham sido considerados na grande maioria dos casos (Yoo et al., 1981; Martínez-Sebastián & Ménsua, 1985; Parkash et al., 1999; Karan et al., 2000; Lyman et al., 2002). Portanto, não existem informações sobre variação ao longo dos segmentos e nem sobre a ocorrência de correlação entre eles. Contudo, a ocorrência de dimorfismo sexual foi observada em várias espécies (Yoo et al., 1981), embora padrões diferentes sejam encontrados. Aparentemente, no subgênero *Sophophora* encontramos fêmeas com mais cerdas que machos e no subgênero *Drosophila* podemos observar o contrário.

Modelos teóricos sugerem que o dimorfismo sexual pode resultar de seleção sexual, competição intra e interespecífica por recursos, ou ambos. Alternativamente, o dimorfismo sexual poderia ser produzido por uma seleção negativa de caracteres específicos de machos, quando

presentes em fêmeas (Kopp et al., 2000). Estudos anteriores obtiveram conclusões divergentes a cerca da relação entre número de cerdas e valor adaptativo em *D. melanogaster* (*veja* Dilda & Mackay, 2002). Uma possível razão para isso é que em *D. melanogaster* a importância da pigmentação abdominal como caráter sexual secundário teria substituído o outrora importante número de cerdas, sendo um alvo mais recente para a seleção sexual (David et al., 2005). Estudos recentes mostram que a perda de caracteres sexuais é comum e pode ocorrer por uma troca de preferência pelas fêmeas (Wiens, 2001).

O objetivo deste estudo foi investigar a variação do número de cerdas abdominais em três níveis diferentes: entre segmentos abdominais, entre sexos, e entre duas espécies distantes de drosofilídeos, *D. melanogaster* e *Zaprionus indianus*. Para cada espécie foram usadas linhagens isofêmeas estabelecidas a partir de populações naturais, o que permite uma análise precisa do dimorfismo sexual (David et al., 2003; 2005) e é capaz de fornecer idéias sobre a variância genética do número de cerdas abdominais.

Os resultados mostraram uma forte interação entre o número de cerdas abdominais e o sexo do indivíduo, e ao mesmo tempo diferenças consistentes entre as duas espécies, sugerindo que o dimorfismo sexual no número de cerdas não é evolutivamente restrito.

Diferenciação entre populações

Como já citado anteriormente, a investigação do nível de variação presente entre populações brasileiras de *Z. indianus* pode ajudar a avaliar o processo de invasão. A diferenciação fenotípica pode aumentar ao longo do tempo como resultado de diferenças genéticas adquiridas com o isolamento entre populações e respostas plásticas a condições ambientais (Sebens, 1987). Supondo que a chegada da espécie invasora ocorreu a partir de um evento único, e que isso se deu muito recentemente, não esperaríamos encontrar populações

altamente diferenciadas na ausência de isolamento. De fato, estudos recentes acerca da diversidade alozímica (Machado et al., in press) e da frequência de inversões cromossômicas (Ananina, comunicação pessoal) mostraram que as populações brasileiras são ainda muito uniformes com relação à composição genética, o que sugere que a expansão da distribuição em território brasileiro se deu a partir de uma única população inicial.

Asas de *Drosophila* são um bom caráter para se estudar variação entre populações, já que diversos estudos mostraram que é uma estrutura capaz de responder à seleção natural. Um fenômeno que pode servir como evidência de que populações estão estabelecidas há tempo suficiente para terem sido selecionadas segundo pressões do ambiente é a ocorrência de clines. O padrão mostrado em geral é que asas maiores são encontradas em latitudes maiores, provavelmente em conseqüência das menores temperaturas observadas nessas localidades (Coyne & Beecham, 1987). Da mesma maneira, a forma da asa também é capaz de responder ao ambiente mostrando padrões geográficos (Bitner-Mathé & Klaczko, 1999a,b; Gilchrist et al., 2000; Hoffmann & Shirriffs, 2002). Como a invasão de *Z. indianus* no Brasil se deu recentemente, é muito provável que não tenha havido tempo suficiente para que padrões geográficos na variação morfológica tenham sido formados, embora adaptações climáticas locais possam ocorrer. Por exemplo, Huey et al. (2000) observaram um cline latitudinal para tamanho do corpo em populações norte-americanas de *D. subobscura* depois de aproximadamente 20 anos do evento de invasão. Um resultado recente mostra que não existe relação significativa entre o comprimento da asa e a latitude para *Z. indianus* na América do Sul (Fig. 1, gentilmente cedida por J. R. David), ao contrário do observado na Índia. No continente africano existe relação entre comprimento da asa e latitude, embora seja mais fraca que na Índia.

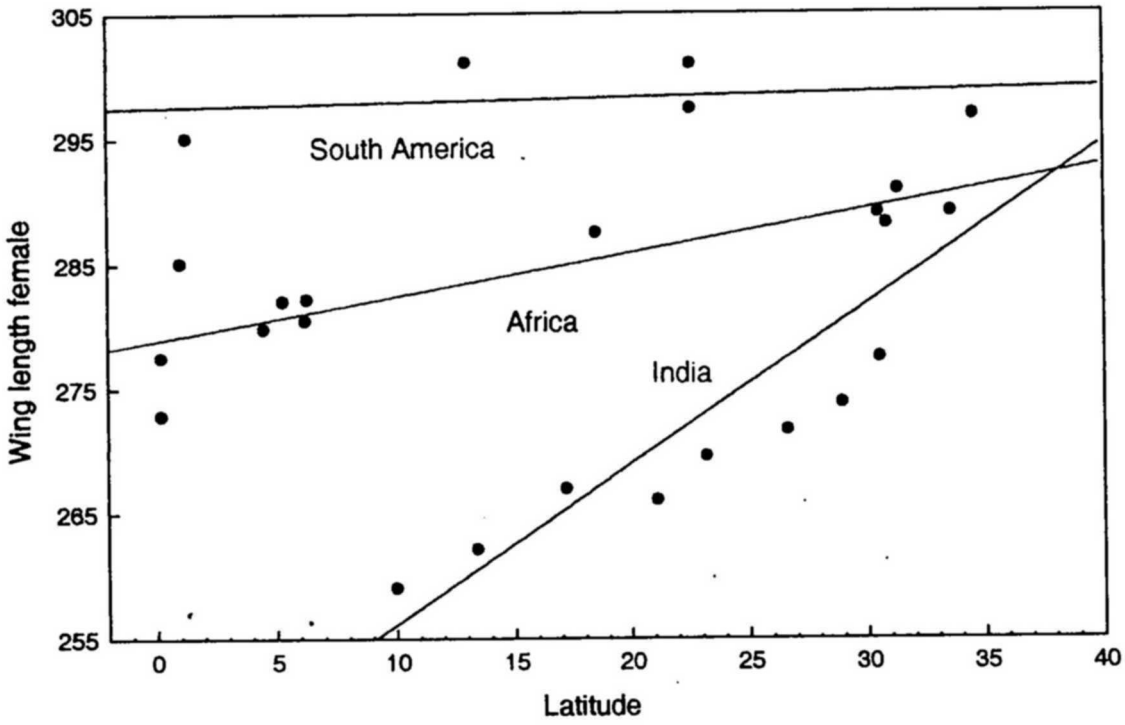

Figura 1: Relação do comprimento da asa de fêmeas de *Z. indianus* pela latitude, em populações da América do Sul, África e Índia. (Figura cedida por J. R. David).

Além das diferenças entre populações, uma abordagem interessante para o estudo de caracteres quantitativos é a quantidade de variação dentro de cada população. Mais ainda, é importante saber o quanto dessa variação é herdável para estimarmos qual a capacidade evolutiva da população, ou seja, qual a possibilidade de que mudanças surgidas e incorporadas na população sejam passadas para gerações seguintes (Falconer & Mackay, 1996).

A herdabilidade (h^2) de um caráter é dada pela proporção da variância fenotípica total (V_P) que é genética aditiva (V_A). Pode-se estimar a quantidade de variância genética aditiva dentro de uma população a partir da covariância fenotípica entre parentes. Uma maneira mais prática é fazendo-se a regressão do valor de um caráter fenotípico entre parentes. Como a herdabilidade é uma propriedade não só do caráter, mas também da população em questão, estimativas para uma

população não podem ser estendidas a outras. Da mesma maneira, estimativas feitas em laboratório e no campo para a mesma população podem ser bem diferentes. Em geral, encontra-se valores mais baixos para a herdabilidade calculada em condições naturais do que para a calculada em laboratório, dado que a instabilidade ambiental é maior e promove uma maior variabilidade fenotípica (Riska et al., 1989; Weigensberg & Roff, 1996; Falconer & Mackay, 1996). Isso ocorre principalmente para caracteres relacionados ao tamanho, cuja variação é freqüentemente composta de um grande componente plástico. Para a forma, valores altos de herdabilidade têm sido encontrados na natureza, mesmo quando a variação fenotípica é maior que no laboratório (Bitner-Mathé & Klaczko, 1999a,b; Hoffmann & Shirriffs, 2002; Moraes & Sene, 2004).

Alguns autores sugeriram uma aproximação da herdabilidade natural através da regressão dos valores dos filhos obtidos em laboratório pelos valores parentais medidos em condições naturais (Coyne & Beecham, 1987; Riska et al., 1989). Um problema dessa abordagem é a possibilidade de ocorrência de interação genótipo-ambiente, ou seja, os genótipos podem responder diferencialmente aos ambientes natural e de laboratório, o que introduziria erro às estimativas de herdabilidade (Falconer & Mackay, 1996; Matta & Bitner-Mathé, 2004). De qualquer maneira, a estimativa obtida com esse método é importante porque fornece um limite inferior para a herdabilidade natural (Riska et al., 1989). Estudos que usaram a regressão entre ambientes diferentes têm encontrado resultados semelhantes (baixa herdabilidade para tamanho e alta herdabilidade para forma) aos obtidos quando filhos e parentais são medidos no mesmo ambiente.

O objetivo do estudo de diferenciação entre populações de *Z. indianus* foi comparar tamanho e forma da asa usando indivíduos provenientes de 10 coletas diferentes, incluindo 6 localidades espalhadas ao longo do centro-leste do Brasil. Para medir tamanho e forma foi usado o método de Morfometria Geométrica. Este método permite uma avaliação quantitativa e

qualitativa de mudanças gerais e localizadas na estrutura em questão, já que é possível recuperar as posições relativas entre os pontos de referência depois das análises estatísticas. A partir de 1983, as grades de deformação semelhantes às construídas por D'Arcy Thompson (1917), passaram a ser quantificadas e submetidas a análises estatísticas. Esta metodologia vem sendo aprimorada por F. L. Bookstein e F. J. Rohlf e vem complementando ou mesmo substituindo a Morfometria Tradicional.

Enquanto a Morfometria Tradicional aplica análises multivariadas a medidas lineares de estruturas e órgãos bem definidos, a Morfometria Geométrica é baseada em pontos notáveis desses órgãos, chamados de pontos de referência (*landmarks*). O uso de pontos de referência é superior ao uso de medidas de distância por dois motivos: a) possibilita analisar todas as direções de variação na forma, enquanto na morfometria tradicional as medidas são tomadas principalmente nas direções paralelas aos eixos de variação da estrutura, b) evita o problema da existência de dependência entre medidas diferentes, que ocorre quando a mesma região está envolvida em mais de uma medida tomada ao longo do mesmo eixo (Monteiro e Abe, 1997; Monteiro & Reis, 1999; Zelditch et al., 2004).

Na metodologia de Morfometria Geométrica o tamanho é medido por um único valor, que é capaz de representar a variação ocorrendo em todas as direções, e que, na ausência de alometria, é independente da variação em forma da amostra (Bookstein, 1991). Para estudar a forma e possibilitar a comparação de grupos, as coordenadas dos pontos de referência devem seguir alguns critérios de alinhamento: 1) transladar as configurações de pontos de modo que os centróides (centro de massa) de todas elas estejam localizados na origem do sistema de eixos; 2) proporcionalizar para que todas tenham tamanho do centróide (raiz quadrada da soma dos quadrados das distâncias entre cada ponto de referência e o centróide) igual a 1 (Goodall, 1991; Rohlf, 1996); e 3) rotacionar para minimizar as diferenças entre cada ponto de referência e seu

homólogo na configuração média. A configuração média, também chamada de consenso, é estimada iterativamente por uma série de sobreposições.

Um conceito importante para entender a maneira com que a forma é estudada é a distância de Procrustes. Quando mais de 2 espécimens estão sendo comparados, uma configuração média é estimada e sobre ela os outros espécimens vão ser alinhados como descrito acima. A forma de um indivíduo pode ser então definida como sua distância de Procrustes (raiz quadrada da soma das diferenças ao quadrado, de cada ponto de referência para seu homólogo na outra configuração em questão) com relação ao consenso, e por isso é um conceito relativo (Rohlf, 1999). Essa métrica é calculada num espaço esférico, onde as formas são representadas como pontos na superfície (Kendall, 1984; Dryden & Mardia, 1998). Para conjuntos de dados reais, as distâncias no espaço de Kendall são equivalentes a distâncias lineares projetadas num espaço tangente (Marcus et al., 2000).

Uma outra maneira de se estudar a forma é usando os escores das deformações parciais e dos componentes uniformes em análises multivariadas. As deformações parciais são obtidas pela projeção das configurações alinhadas sobre as deformações principais. Estas derivam de uma matriz de energia de deformação, calculada como função das distâncias entre os pontos na configuração consenso, assumindo que a estrutura sobre a qual a configuração de pontos está colocada é uma placa de metal superfina, de dimensões infinitas. Quanto maior a distância, menor a energia necessária para deformar a placa de metal em vista do ajuste das configurações. As deformações parciais descrevem a variação da forma em diferentes escalas, desde mudanças globais (componentes uniformes) até mudanças localizadas (Bookstein, 1989). Como essas variáveis são muito dependentes do número e localização dos pontos na configuração consenso, elas não têm significado biológico quando analisadas separadamente. Portanto, os escores das deformações parciais podem ser submetidos a uma análise de deformações relativas (RWA), que

redistribui a variação da amostra, assim como numa análise de componentes principais. Os escores das deformações relativas podem ser usados em análises estatísticas univariadas.

Em resumo, três aspectos importantes da biologia da espécie invasora *Z. indianus* foram estudados durante minha pesquisa de doutorado, incluindo questões sobre a habilidade de dispersão e capacidade adaptativa, desenvolvimento e dimorfismo sexual, e diferenciação dentro e entre populações. Os resultados contribuem para uma caracterização do estado inicial da invasão.

1. Tolerância Térmica

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Male sterility thresholds in a tropical cosmopolitan drosophilid, *Zaprionus indianus*

L.O. Araripe[a,b], L.B. Klaczko[a], B. Moreteau[b], J.R. David[b,*]

[a] *Departamento de Genética e Evolução, Instituto de Biologia, Universidade Estadual de Campinas, Unicamp, CP: 6109, Campinas, SP 13083-9790, Brazil*

[b] *Laboratoire "Populations, Génétique et Evolution", UPR 9034, bât. 13, CNRS, Gif-sur-Yvette Cedex 91198, France*

Abstract

When grown and kept at extreme constant temperatures, *Zaprionus indianus* males are sterile and do not produce viable sperm. This phenomenon, already investigated in two *Drosophila* species, might be a general feature in drosophilids. On the high temperature side, sterility thresholds were similar in *Z. indianus* and *D. melanogaster*. On the low temperature side, *Z. indianus* was much more sensitive to cold. This might explain why *Z. indianus* is restricted to tropical and subtropical climates, while *D. melanogaster* also proliferates in temperate places. After returning to a middle, permissive temperature, males recovered fertility. The time to recover was always longer than in *D. melanogaster*, and the number of progeny much smaller in all cases. Such differences may be due to anatomical and functional divergences, and specially the longer sperm length in *Z. indianus*.

Keywords: Thermal plasticity; Cold and heat stress; Development; Reproduction; Sperm size; *Zaprionus indianus*; Insect

1. Introduction

For ectotherm species, temperature appears as a major environmental factor for explaining the distribution and abundance of animals (Andrewartha and Birch, 1954; Precht et al., 1973; Cossins and Bowler, 1987; Leather et al., 1993). In most cases a physiological trade-off seems to exist between cold and heat tolerance. Species living at high latitudes may be classified as cold tolerant but heat sensitive, while species restricted to tropical places exhibit a cold sensitivity but a heat tolerance.

Thermal adaptation is generally investigated by measuring the thermal sensitivity of a diversity of fitness related traits, such as viability, rate of development and offspring production (Hoffmann et al., 2003). But new criteria may also be considered, such as the time required for recovering after a cold-induced chill coma (David et al., 1998; Gibert et al., 2001; Hoffmann et al., 2002) or male sterility thresholds symmetrically observed at low or high developmental temperature (Chakir et al., 2002).

In *Drosophila melanogaster*, male sterility after a development at 30°C, with a possible recovery after a return to a medium temperature, was described in 1971 (David et al., 1971). A quite similar phenomenon is sterility at low temperature, which was described 3 years later (Cohet, 1973). At that time, both phenomenas were considered as physiological oddities, not interesting from an evolutionary point of view, and not worth further investigations. Only in 2002 a comparative analysis on the two cosmopolitan sibling species, *D. melanogaster* and *D. simulans*, revealed a clear-cut difference (Chakir et al., 2002). The low-temperature sterility threshold was about 12°C in *D. simulans* and 13°C in *D. melanogaster*. On the high-temperature side, a complete sterility was observed at 28°C in *D. simulans* and 30°C in *D. melanogaster*. In other words, *D. simulans* seems more tolerant to cold but more sensitive to heat than its sibling.

*Corresponding author. Tel.: +33-1-69-823613; fax: +33-1-69-070421.

E-mail address: david@pge.cnrs-gif.fr (J.R. David).

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This observation raises two general questions. Is male sterility at extreme temperatures a general feature in *Drosophila*? Are variations in sterility thresholds a significant component of the thermal niche of a species?

In the present paper we focus on a tropical drosophilid, *Z. indianus*, which is native from the Afrotropical region but has recently colonized the South American continent (Vilela, 1999; Goñi et al., 2001; Tidon et al., 2003). Biogeographical data show that this species cannot survive at high latitudes and that it is restricted to warm tropical and subtropical climates. The thermal range for viability of *Z. indianus* is known to be 12–32°C (Karan et al., 1999) and, in this respect, it is not very different from that of *D. melanogaster* (11–32°C) (Pétavy et al., 2001). Such a small difference seems insufficient to explain the clear-cut difference in the geographic distributions of the two species. One possibility is that other fitness-related traits might reveal a larger difference, and here we analyze the relationship between growth temperature and male fertility. We found that both high and low temperatures experienced during development induced a reversible sterility. Interestingly, males grown at 15°C were found to be 100% sterile, a difference of almost three degrees with *D. melanogaster*. This cold sensitivity of male reproductive function may explain why *Z. indianus* cannot permanently colonize temperate countries.

2. Materials and methods

2.1. Strains investigated

Most experiments were done on a Brazilian mass strain, collected in 2001 in the Tijuca tropical forest, in Rio de Janeiro, and founded by about 20 pairs of wild living adults. Some comparative observations were done on other African or Indian strains, kept in the Gif laboratory for several years.

2.2. Experimental cultures

The Tijuca strain was raised at 21°C under a LD 16-8 photoperiod, in 250 ml bottles, on a corn-meal-sugar food seeded with live yeast. Adults were transferred to fresh vials every 4–5 days. Immediately after, groups of about 100–200 young larvae were taken with a spatula and transferred to experimental smaller bottles (125 ml) containing the same medium, seeded with live yeast and supplemented with a piece (about 5 g) of a high nutrient, killed yeast medium (David and Clavel, 1965). These bottles were then transferred to experimental, constant temperatures. We investigated four low temperatures (14–17°C) and four high temperatures (28–31°C). A high thermal stability was achieved by using small incubators (about 225 l) with a permanent ventilation, themselves kept in temperature controlled rooms, either at 6–7°C for the low temperature incubators, or at 19–20°C for the high temperature side. We also produced control flies, permanently kept at a medium temperature (21°C). After emerging at various growth temperatures, adult flies were transferred to fresh food and kept at the same temperature. Growth temperatures of 21°C and also 15°C were obtained not in small incubators but in thermally controlled rooms, with a lower precision.

2.3. Presence of sperms in seminal vesicle

The anatomy (Fig. 1) and reproductive physiology of *Z. indianus* are quite different from what is known in *D. melanogaster* (Ashburner, 1989). Sperms are much longer in *Z. indianus* than in *D. melanogaster* (about 5 mm vs. 1.8 mm). As a consequence, both the testis and the seminal vesicles are longer (see Fig. 1). Also the age at maturity is delayed: fertile copulations at 21°C are obtained after 4–5 days (Karan et al., 1999) against 1 day in *D. melanogaster*.

The presence of mobile sperm in the seminal vesicle was checked after a dissection of the male reproductive organs in *Drosophila* saline solution, and then piercing the seminal vesicles with tiny insect minutia pins. When sperm is present, a fibrous mass can be seen with a binocular microscope, and even the mobility of the sperm flagella is visible. In case of sterility, nothing is found in the seminal vesicles, which keeps a small diameter.

The rate of sperm production and age at maturity are strongly affected by temperature. For all the high-temperature treatments (including 21°C) dissected

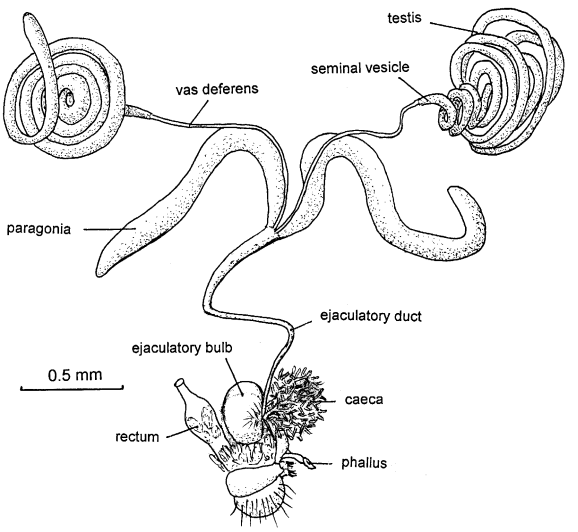

Fig. 1. Anatomy of male reproductive organs of *Zaprionus indianus*. Testes and seminal vesicles are orange–yellow. Other parts are not pigmented.

males were aged of at least 1 week. For the low temperatures, the age at dissection was always superior to 2 weeks.

2.4. Fertility recovery and progeny number after a transfer to 21°C

Young males, aged 1 day, grown at various experimental temperatures, were isolated without anesthesia and brought to the permissive temperature of 21°C. Each was mated with three normal, mature virgin females, grown at 21°C and aged 3–4 days, in ordinary culture vials (20 ml) containing a corn-meal sugar food. Then, each such group of one male and three females was transferred daily to a new vial (a 2-day interval was used for the weekend). All vials were kept at 21°C and examined after about a week. A male was considered as fertile when at least one larva was observed in the culture vial. After about 20 days, each vial was again examined and emerged adults counted. When laying parents remained 2 days in the same vial the progeny number was corrected to a daily rate, i.e., divided by two.

3. Results

3.1. Fertility of mass cultures kept at constant temperatures

Adult flies grown at various constant temperatures were kept at the same temperature and transferred regularly to fresh bottles. These bottles were eventually examined for the presence of progeny. Such experiments, repeated in each case several times, for a total number of adults overpassing 100, led to clear-cut conclusions. On the low temperature side, progeny were never observed at 14°C or 15°C, even after a month, but always at 16°C and 17°C. On the high temperature side progeny never showed up at 30°C and 31°C, but were present at 29°C and 28°C. In all cases, females produced eggs and, when mated with control, 21°C reared males, rapidly produced an offspring. In other words, the lack of progeny is attributable to male sterility only.

Several other geographical strains of *Z. indianus* from tropical Africa (Cotonou, Sao Tomé and Cape Town) and India (Delhi) were also grown, in a similar way, at the critical temperatures of 15°C and 30°C. In all cases progeny were never observed: these threshold temperatures for male complete sterility appear as a general property of the species.

3.2. Presence of sperm in seminal vesicles

Males grown and kept at various constant temperatures were dissected and their genital tract examined for the presence of sperms in seminal vesicles. Age of dissection was at least 1 week for the high temperatures (21°C and above), and more than two weeks for the low temperatures (14–17°C). Proportions of fertile males are given in Table 1, and shown graphically in Fig. 2.

Table 1
Presence of mobile sperms in the seminal vesicle of males grown under different temperatures

Treatments°C	Number of males dissected	Males with mobile sperm(%)	Males without sperm (%)	Abnormal testes
14	39	0	38	1
15	49	8	41	0
16	51	20	30	1
17	50	40	10	0
21	54	47	3	4
28	53	49	1	3
29	61	20	30	11
30	53	0	46	7
31	58	2	48	8

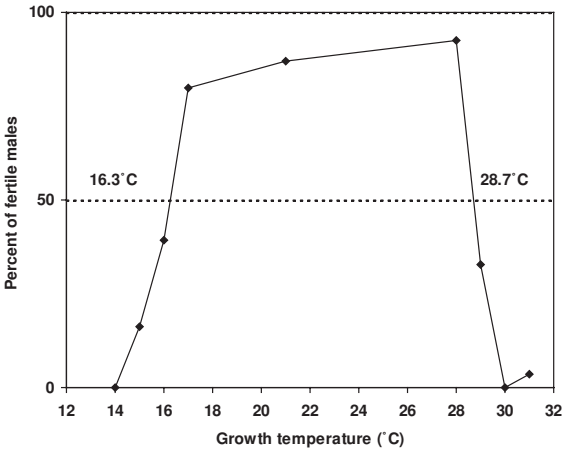

Fig. 2. Variation of male fertility as a function of growth temperature in *Z. indianus*. Temperatures that produce 50% of sterile male (MST-50) are estimated graphically.

As expected, we found very few or no male producing sperm at extreme, low or high temperatures, while a high fertility was observed in the middle of the range. The overall curve is similar in shape to what was already found in *D. melanogaster* and *D. simulans* (Chakir et al., 2002), with a kind of plateau between 17°C and 28°C and very sharp decreases at low and high temperatures. Some males with abnormal testes were observed at most temperatures (Table 1). These abnormalities corresponded to an atrophy or absence of the testes. They were especially common at high temperatures, on average 15% between 29°C and 31°C.

Fig. 2 permits a graphical estimate of the temperatures that produced 50% of sterile males (male sterility

temperature 50%, or MST-50). These temperatures are estimated to be 16.3°C and 28.7°C for the low and high sides, respectively. The graph also shows that the slope is steeper on the heat side than on the cold one.

A quite surprising result is that, at 15°C, we found by dissection about 15% of the males with sperm, while by the progeny test (previous section) all males were scored as completely sterile. There are two possible explanations for such a discrepancy. One is that, for this experimental condition, temperature was not conveniently regulated. As stated before, development occurred in a large climatic room, which was less stable than a small incubator. So it may be possible that, instead of being exactly 15°C, the real average temperature was in fact 15.2–15.3°C. The alternative hypothesis is that, although some males did produce a few sperms in their seminal vesicles, these sperms could not be efficiently transmitted to the females and, hence, the eggs could not be fertilized. This interpretation is probably only valid for the two males which, grown at 31°C, were scored as producing sperm (see Table 1).

3.3. Fertility recovery at 21°C

Males grown at various temperatures were transferred individually to 21°C and mated with mature virgin females. Results for progeny production are given in Fig. 3 and statistical comparisons are done in Table 2.

Control males, grown and kept at 21°C (Fig. 3A), all produced offspring. Interestingly, no progeny was observed at day 4, but almost all males produced an offspring at day 5. We may conclude that, at 21°C, it takes 5 days for the male to reach sexual maturity. All males remained fertile for about two weeks, but after that some experimental vials, still containing one male and three females, failed to produce larvae.

After a development at 17°C, all males also produced progeny, but the average curve (Fig. 3A) was somehow less regular. Also the age for reaching maturity was slightly longer than for 21°C males (6.58 vs. 5.15 days, Table 2). Still less favorable results were obtained at lower temperatures. For 16°C, the first progeny was observed at an average age of 9.45 days and only 83% of the males became fertile. For 14°C males, the average age for progeny production was 11.50 days and only half of them recovered fertility. Data for a growth temperature of 15°C are not shown because, when the experiment was done, the climate room underwent a breakdown and the temperature overpassed 20°C for more than 3 days.

On the high-temperature side (Fig. 3B), males grown at 28°C were normally fertile, although only 75% of them produced offspring, and also the age at first reproduction was slightly delayed. As shown in Table 1, about 15% of these males were expected to have

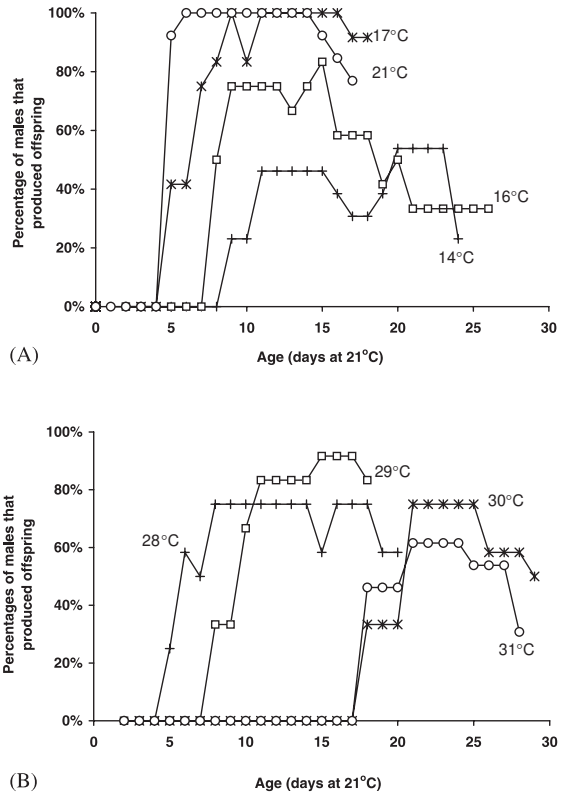

Fig. 3. Recovery of male fertility after a transfer to 21°C. Growth temperatures are indicated for each curve. (A) results for low developmental temperatures and for 21°C. (B) results for high developmental temperatures.

abnormal testes and remain completely sterile. At 29°C, more males (92%) were fertile but the age at maturity was longer (9.64 days). Males grown at 30°C and 31°C produced similar data: a fertility percentage not exceeding 75% and a late progeny production at about 20 days.

3.4. Number of progeny at 21°C

As stated in methods, we counted the number of flies produced by males after they being transferred at 21°C with normal mature females. With this procedure, progeny number will reflect sperm production by males and sperm utilization by females. We first examined the daily fecundity curves, which were quite irregular due to the small number of males studied. Mean daily progeny number over the period of fertility are given in Table 2. Males grown at 28°C produced on average 24.5 progeny per day. A similar value (23) was obtained by the 21°C males. For other growth temperatures, lesser values were obtained: on average 10 per day for males grown either at 14°C or at 30–31°C.

Table 2
Fertility parameters for males grown at various temperatures and transferred to 21°C after emergence, each with three mature virgin females grown at 21°C. Means±s.e. are given

Treatments	n	Fertility %	Mean age at first fertility (days)	Daily fecundity(days)	Calculated age at maturity(days)	Slope (cumulative curve)	R^2
14°C	13	54	11.5 ± 1.0^{b}	9.9 ± 0.8^{a}	$10.1\pm1.1^{a,c}$	6.8 ± 2.4^{a}	0.93 ± 0.02
16°C	12	83	9.5 ± 0.8^{b}	$13.7\pm1.6^{a,c}$	$8.2\pm0.7^{a,b,c}$	15.1 ± 1.9^{a}	0.96 ± 0.01
17°C	12	100	6.6 ± 0.5^{a}	$19.7\pm1.8^{b,d}$	$6.2\pm0.8^{a,b}$	21.6 ± 2.4^{b}	0.96 ± 0.01
21°C	13	100	5.2 ± 0.2^{a}	22.9 ± 1.4^{b}	$4.7\pm0.4^{a,b}$	24.0 ± 1.7^{b}	0.96 ± 0.01
28°C	12	75	6.3 ± 0.4^{a}	24.5 ± 1.5^{b}	$6.3\pm0.5^{a,b}$	25.6 ± 3.7^{b}	0.97 ± 0.01
29°C	12	92	9.6 ± 0.6^{b}	$17.9\pm1.7^{b,c}$	$9.8\pm0.4^{a,b,c}$	18.8 ± 2.5^{b}	0.95 ± 0.02
30°C	12	75	20.0 ± 0.6^{c}	9.8 ± 0.7^{a}	18.6 ± 0.7^{d}	9.4 ± 2.3^{a}	0.92 ± 0.02
31°C	13	62	19.2 ± 0.6^{c}	$14.4\pm0.8^{a,c,d}$	17.4 ± 0.4^{d}	14.2 ± 2.6^{a}	0.94 ± 0.02

n: number of males studied; fertility %: percent of males which produced progeny. For each male, a linear regression was calculated between age and the cumulated number of progeny. The good fit of the linear regression is shown by high R^2 values. The age at maturity was calculated using the regression parameters. The average slopes of regression are also given for each temperature.
^{a,b,c,d}Indicate significantly different means after testing by Tukey post hoc test ($p< 0.05$).

With this experimental procedure (one male kept with three normal females) it was previously found in *D. melanogaster* and *D. simulans* (Chakir et al., 2002) that the number of progeny increased regularly over time. We examined the data for *Z. indianus* and found variable results. A significant increase in progeny number over time was found in two cases only, for males grown at 28°C and 29°C (Fig. 4). For all other temperatures, progeny number did not increase significantly over time (this phenomenon is illustrated in Fig. 4A for 17°C and 21°C grown males).

For a general comparison of all temperature data, we decided to consider the cumulative curves instead of the daily production (Fig. 5). In each case, the individual curves were conveniently adjusted to a linear model, with R^2 average values always superior to 0.90 (see Table 2). Each male was characterized by the slope and the intercept of the regression. The slope is an estimate of the daily production, assuming it remains constant over time. The values found (Table 2) exhibit significant differences according to temperature, being maximum for 17°C, 21°C and 28°C, and minimum for extreme low

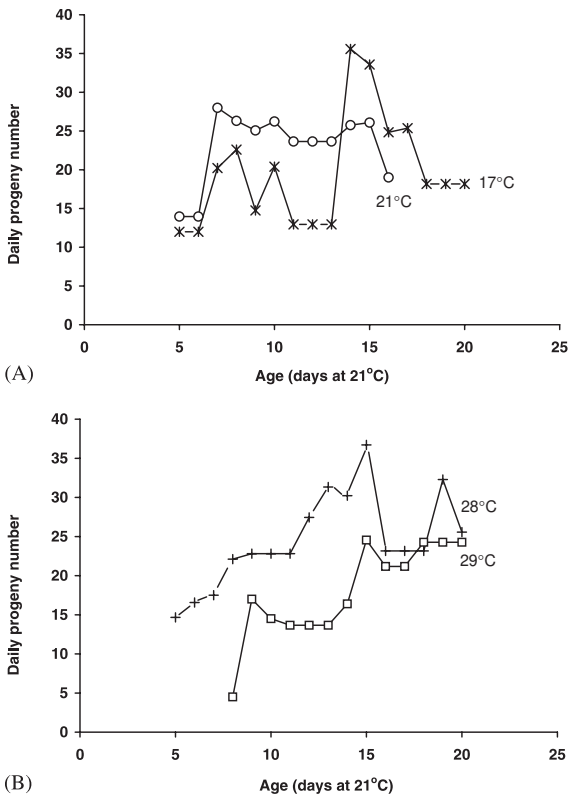

Fig. 4. Daily progeny number (mean). (A) data for 17°C and 21°C used as examples. (B) data for 28°C and 29°C (the only two cases where a significant increase in progeny number over time was observed). Only males which recovered some fertility were used for calculations.

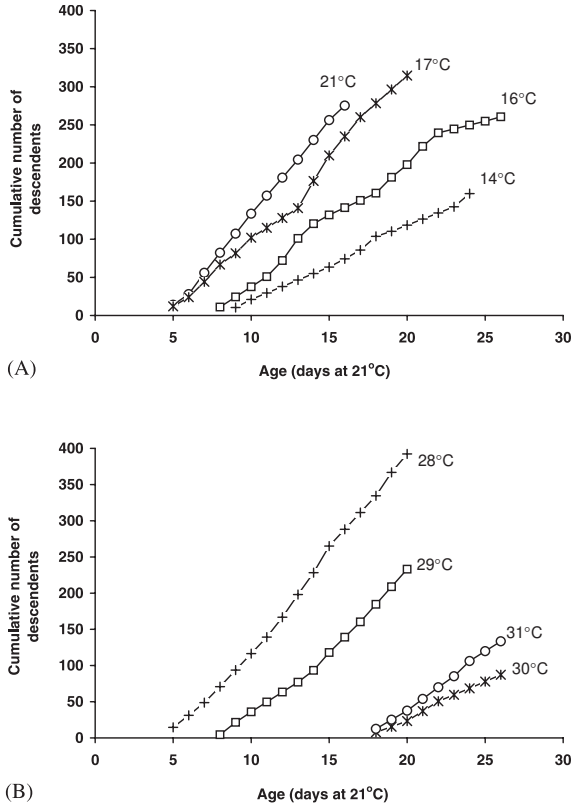

Fig. 5. Cumulative number (mean) of descendents produced per day for each temperature studied. (A) data for low and optimal (21°C) temperatures, (B) data for high temperatures.

or high temperatures. This pattern of variation is similar to that observed for average daily production. Using the intercept values, we calculated for each male the age at which the progeny number would be zero, in other words the age at which fertility begins. This calculated age at maturity is minimum for 21°C and increases for lower or higher growth temperatures. The two values, observed and calculated age at maturity, exhibit some differences (Table 2) but their variation is basically similar and they are strongly correlated ($r = 0.97$).

Whichever the way they are calculated, the two traits (age at maturity and daily sperm production) are negatively correlated. An early reproduction under favorable thermal conditions is accompanied by a high rate of sperm production and vice versa. For the males sterilized by a growth at an extreme temperature, the recovery at a middle temperature is possible, but is never complete.

4. Discussion and conclusions

Our results may be discussed from a physiological point of view, but also for their ecological and evolutionary significance. In each case, data on *Zaprionus* need to be compared with what is already known (Chakir et al., 2002) in two other cosmopolitan species, *D. simulans* and *D. melanogaster*, especially with the latter.

We found that, in *Zaprionus*, both low and high temperatures induced a male sterility, while female egg production, although not precisely investigated, was not much affected. There are however significant differences between *Z. indianus* and the two *Drosophila* species. A major difference concerns the age at maturity under normal conditions: while *D. melanogaster* males produce fertile sperm and offspring on their second day of life, a fairly long delay (about 5 days) is needed in *Zaprionus* (Karan et al., 1999; and present study). This delayed maturity seems correlated with a greater sperm length (Fig. 1) and thus a longer time for sperm elongation (Pitnick et al., 1995; Simmons, 2001).

Sterilizing effects of extreme growth temperatures may be appreciated either by estimating an absolute threshold (no F1 in cultures kept at the same temperature) or a relative threshold producing 50% of sterile males (MST-50). Absolute thresholds in *Zaprionus* are 15°C and 30°C, and relative thresholds (Fig. 2) are 16.3°C and 28.7°C. They are much higher than in *D. melanogaster* on the low temperature side (12°C and 13.2°C), but similar at high temperatures (30°C and 29.1°C). Remarkably, males of both *Z. indianus* and *D. melanogaster* are fully sterile at 30°C.

Transferring freshly emerged males from a sterilizing to middle, permissive temperature allows a recovery of fertility after some delay. In *D. melanogaster*, recovery times are quite similar when considering the effects of cold or heat, and also recovery is much longer for very extreme growth temperatures (Chakir et al., 2002). For example, recovery takes much more time after a development at 31°C than after a development at 30°C. Such is not the case in *Zaprionus*. Firstly, we found that recovery was much faster for a low sterilizing temperature (11.5 days for 14°C) than for a high temperature (20 days for 30°C). Secondly, we found similar recovery times for different extreme temperatures, for example 14°C and 16°C, or 30°C and 31°C. Such differences suggest that the harmful (not known) effects of extreme temperatures on spermatogenesis are not exactly the same in *D. melanogaster* and *Z. indianus*.

A last interesting comparison concerns the progeny number. In *D. melanogaster*, with the same protocol of one male and three females, it was found that the daily offspring number increased over time (Chakir et al., 2002), presumably reflecting an increase of sperm production by the male, accompanied by an efficient storage in females. For example, for a normal, 21°C grown male, the average daily offspring production increased from 40 up to 160 descendents in 10 days. Results were quite different for *Z. indianus*, and

especially the daily progeny production did not increase over time. As a consequence, calculations and statistical comparisons were done on linear, cumulative curves. The slope of these curves estimates the daily progeny production, which in the best cases (21°C and 28°C grown males) remained around 25. Again, this low reproductive potential may be related to the larger sperm size and presumably a much lesser number (Simmons, 2001). After a development at extreme, low or high temperatures, the progeny number remained much lower, in spite of the fact that calculations were done only on males that recovered fertility. In that respect, the recovery was better from high-temperature development (about 12 offspring a day for 30–31°C) than from low temperatures (daily fecundity less then 7 for 14°C). This effect on progeny number is in opposite direction compared to that observed for the time of recovery.

From an ecological point of view, both *Z. indianus* and *D. melanogaster* exhibit quite similar thermal ranges in development, with a midpoint (optimum ?) at 22°C and 21°C, respectively. It is only on the cold side that a major difference is observed, with a higher temperature of about 3°C in *Z. indianus* for both the absolute threshold and the MST-50. In this respect, *Zaprionus* appears clearly more sensitive to cold than *D. melanogaster*, and this ecophysiological difference may explain why *Z. indianus* cannot colonize temperate places and remains restricted to tropical and subtropical climates (Chassagnard and Kraaijeveld, 1991; Goñi et al., 2001).

It is generally assumed, in demographic investigations, that the rate of increase in number (r parameter) depends on the rate of female gamete production. Such might not be the case in *Zaprionus*. The ovariole number in female is slightly superior to 40 (unpublished result). If we assume that, at 25°C, each ovariole produces one egg per day, the average daily progeny production should be 40. This is a conservative estimate since, in *D. melanogaster*, each ovariole in well fed females produces about two eggs a day (Chakir et al., 2002). In the present work, we found that the maximum progeny production by a male mated with 3 normal females was about 25. In other words, sperm production might be the limiting factor for demographic expansion of *Zaprionus*.

Finally, from an evolutionary perspective, *Z. indianus* is the third drosophilid for which we know that extreme temperatures, before producing a lethal effect, induce a male specific sterility. Whether this phenomenon is general in drosophilids and other insects is not known. There is however some evidence that, at least on the heat side, sterile males are observed in other species, for example at 30°C in *D. buzzatii* (Vollmer et al., 2003 in press) and at 25°C in *D. subobscura* (unpublished results). We already know that, among *Drosophila* species, the thermal range is highly variable and reflects a thermal adaptation, comprised between 6°C and 26°C in the cold tolerant, temperate *D. subobscura* (Moreteau et al., 1997) and between 16°C and 32°C in the circumtropical *D. ananassae* (Morin et al., 1997). We now suggest that, besides the lethal temperature limits, male sterility thresholds should also be considered and might be, in some cases, more informative for understanding the biogeographical distribution of a species.

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2. **Caracteres Morfológicos**

Abdominal bristle number in two cosmopolitan drosophilids: divergent sexual dimorphism in a modular trait.

L.O. Araripe[a,b], L.B. Klaczko[a*], B. Moreteau[b], J.R. David[b]

[a] *Departamento de Genética e Evolução, Instituto de Biologia, Universidade Estadual de Campinas, Unicamp, CP: 6109, 13083-9790 Campinas, SP, Brasil.*
[b] *Laboratoire "Populations, Génétique et Evolution", UPR 9034, bât. 13, CNRS, 91198 Gif-sur-Yvette Cedex, France*

Abstract

The number of neurosensory bristles on abdominal sternites of *Drosophila* is a classical and most investigated trait for quantitative genetic studies. However, the developmental pattern expressed on successive segments has remained so far a neglected field. In this paper, we explore three aspects of this general problem: we compare two distantly related species, *Drosophila melanogaster* and *Zaprionus indianus*; we investigate the variation along the antero-posterior (AP) axis, and we analyse the sexual dimorphism. An isofemale line experimental design provided information on mean values, within and between line genetic variability, as well as on phenotypic and genetic correlations.

Antero-posterior variations could be analysed from segment 2 (A2) to 7 (A7) in female, and A2 to A5 in male. In *D. melanogaster,* males and females showed parallel curves of variation. In *Z. indianus* females the variation was stable along the abdomen, while in males an important and almost linear increase was found from A2 to A5, accounting for a significant sexual dimorphism. The sexual dimorphism was further analysed by considering either the female-male correlation or the female/male ratio. These results suggest that sternite bristle number is determined by several developmental genetic systems: one is acting along the AP axis and may be associated to a

gradient of genetic control; another is acting in the same way on most segments of both sexes; finally, genes with specific sex effects are acting on A7 in females of both species, and on A5 in *Z. indianus* males. The overall architecture of female and male abdomen seems to be constrained by the organization of the reproductive system. Conversely, the difference between species suggests that the sexual dimorphism at the bristle number level is not evolutionarily constrained.

Running Title: Abdominal bristle number in two drosophilids

Key words: *Drosophila melanogaster; Zaprionus indianus;* quantitative traits*; sexual dimorphism.*

1. Introduction

The number of neurosensory bristles on the thorax sides or on the abdominal sternites of adult *Drosophila melanogaster* has been used in numerous quantitative genetic studies for over 50 years (Falconer and Mackay, 1996). There are at least four main reasons for explaining this scientific interest: (1) although quite variable among individuals, these numbers are easily accessible with essentially no measurement error, (2) there is a reasonable amount of information about major mutations affecting bristle number (Lindsley and Zimm, 1992), (3) the developmental basis of the phenotypes are quite well understood (Mackay, 2001; Brakefield, 2003), (4) recent investigations have revealed a diversity of QTL (quantitative trait loci) in natural populations (Shrimpton & Robertson, 1988a,b; Lai et al., 1994; Mackay, 1995; True et al., 1997; Long et al., 1998, 2000; Nuzhdin et al., 1999; Kopp et al., 2000; Dilda & Mackay, 2002).

Although abdominal segments share the same basic architecture with a dorsal plate (the tergite) and a ventral plate (the sternite), and thus can be considered as a modular trait, it is strongly affected by sex. In the female, seven segments are easily recognized, each harboring a visible tergite; sternites with abdominal bristles are visible on segments A2 to A7. In the male, on the other hand, only six tergites are visible (A1 to A6) and the last two are completely black (hence the name of *melanogaster*). The genetic basis of this secondary sexual character has been recently unraveled (Kopp et al., 2003). In females, all segments exhibit the same pigmentation pattern, with an anterior black stripe and a posterior yellow part. The extension of the black part is genetically very variable and is also very plastic according to the developmental temperature (David et al., 1990; Gibert et al. 1998b; Kopp et al., 2003). A recent investigation (David et al., 2005) analyzed the shape of the reaction norms (the variation of the black surface of each segment) according to growth temperature, and found significant differences among all segments. Also, the genetic correlations were always very high between adjacent segments (e.g. A2.A3 or A6.A7), but decreased to zero when more distant segments (e.g. A2.A7) were compared. These results suggest that, although homeotic genes are responsible for the differentiation of abdominal segments, there might be a concentration gradient acting to generate a different genetic architecture and hence, a different phenotypic response for each tergite.

By contrast, the variability among abdominal sternites and their bristle number has remained less investigated, quantitative genetic studies and selection experiments have in most cases considered only one or two successive segments (Yoo et al., 1981; Martínez-Sebastián & Ménsua, 1985; Parkash et al., 1999; Karan et al., 2000; Lyman et al., 2002). Indeed, and more surprisingly, when both sexes have been selected, different segments were considered according to sex, for example the sum of bristles on the 5^{th} and 6^{th} segments in females and the sum of 4^{th} and 5^{th} in males (Frankham, 1977). Data on *D. melanogaster* have shown that males harbor on

average less abdominal bristles than females. However, this does not seem to be a general case. Yoo et al. (1981), comparing bristle number on segment 4 in various species, found that in the *Sophophora* subgenus, males had less bristles than females, but that the reverse could be true in the *Drosophila* subgenus.

The present work was undertaken to extend our knowledge on the pattern of abdominal bristle variation from comparisons in three different levels: among abdominal segments, between sexes and between two distantly related drosophilids, *D. melanogaster* and *Zaprionus indianus*. The first is the reference species for *Sophophora*, while the genus *Zaprionus* is close to species in the *Drosophila* subgenus (Yotoko et al., unpublished). Both species are invasive and cosmopolitan, and *Z. indianus* has recently invaded the South American continent. For each species, an isofemale-line experimental design (David et al., 2005) was used for two natural populations, allowing a diversity of comparisons. This methodology also provides some insight into the genetic architecture of abdominal bristle number and permits a precise analysis of sexual dimorphism (David et al., 2003).

Our data evidenced a strong interaction between abdominal segments and sex, but also consistent differences between the two species, suggesting that the sexual dimorphism at the bristle number level is not evolutionarily constrained.

2. Material and Methods

Origin of samples

Individuals of *Z. indianus* were collected in Cape Town (South Africa) in spring 2002 and females were used to establish isofemale lines. Similarly, wild *D. melanogaster* were collected in Draveil, a locality about 30 km South of Paris (France) in October 2002. These lines were kept

for a few generations at a temperature of 20-22°C and 12 of them were randomly taken in each species. For the production of experimental flies, groups of 10 pairs were randomly taken from each line and used as parents. These parents oviposited for a few hours in vials containing a high nutrient, killed yeast food (David & Clavel, 1965), which at least in *D. melanogaster*, prevents the effects of crowding upon adult morphometry (Karan et al., 1999). In the case of *Zaprionus*, previous investigations showed that development was much more sensitive to larval crowding, and especially that, when too numerous, the mature larvae tended to move out of the vial for pupation and often died out from desiccation. So we controlled more precisely the density in each vial by removing egg and young larvae one or two days after oviposition. Overall, the number of adults from an experimental vial was less than 50 for *Z. indianus*, while it was generally around 100 in *D. melanogaster*. After removal of the parents, experimental vials were incubated at 25 $\pm$ 0.1°C.

Morphological aspects and bristle counting

In *D. melanogaster,* the development of abdominal bristles, which are mechanoreceptors, has been well studied (Kopp et al., 2000; 2003). In the female, seven segments are easily recognized (see Fig. 1), and bristles are visible on sternites A2 to A7. A similar pattern is observed in the female of *Z. indianus* (Fig. 1). In the male of *D. melanogaster,* on the other hand, only six tergites are visible, the last two (A5 and A6) being completely black. On the ventral side, five sternites are recognized, but A6 is deprived of bristles, which can be counted on four successive segments only (A2 to A5). The same situation occurs in the male of *Zaprionus,* except for a difference, sternite 6 is not visible (Fig. 1).

There are variations in the shape and size of the sternal plates of the successive segments, but they are difficult to quantify. In all cases, their shape is more or less rectangular, except the

sternite 7 in females (Fig. 1). This sternite presents two lobes, which extend on both sides of the ovipositor so that a right and left half can be easily identified.

After emergence, adult flies were transferred to fresh food and aged 3-4 days. They were then etherized and kept in a preserving liquid made of 70% ethanol (80%), glycerol (10%) and acetic acid (10%). Abdominal bristles were easily observed and counted on these flies, except for A2. This segment is partially covered by the coxae of the third pair of legs, and in most cases the legs had to be removed for a convenient observation.

Data analysis

Basic statistics (mean and standard error) were calculated for each segment in males and females, and also for the sum of A2 to A5, a trait that is common to both sexes. Differences between segments, sexes and species were investigated with ANOVA or *Student's t* test.

With an isofemale line design, the variability of abdominal bristles can be analyzed at two levels: the within-line variance, which reflects mostly an environmental component, and the between-line variance, which reveals genetic variation among lines (David et al., 2003). We considered the within-line variability as a specific trait (e.g. Moreteau et al., 2003, on sternopleural bristles), and its relationship with mean values variation was eliminated by considering the coefficient of variation (CV). Both components of variance (within and between) were used to calculate an intraclass correlation, which is akin to a heritability value (David et al., 2003). We also considered the genetic CV, which characterizes the capacity of a population to change under selection, and may be called evolvability (Houle, 1992).

Correlations (Pearson's r coefficient) were analyzed at the within-line level. For this approach, we considered the correlations among different segments of the same individual (Via,

1984; Gibert et al., 1998; Kopp et al., 2003) and calculated the within-line means of the coefficients.

The sexual dimorphism was also quantified. A difference related to sex is a frequent observation in quantitative genetics, and QTL analyses often show that the genetic architecture is not the same for males and females. Sexual dimorphism for any trait can be described by considering either the female-male (F – M) difference or the F/M ratio. It is currently considered that a ratio may be a better descriptor of dimorphism, since it eliminates the effect of differences in mean (Reeve & Fairbairn, 1999; David et al., 2003). Thus, for the sake of simplicity, we consider here only the ratio.

For estimating the heritability of sexual dimorphism, we used the same artifice as David et al. (2003) for body size. The variability at the level of individual flies was calculated by dividing, in each line, the value of each female by the mean value of their brothers. With that method, an intraclass correlation coefficient was calculated for each segment. At the line-mean level, the ratios were also used for comparing segments and species.

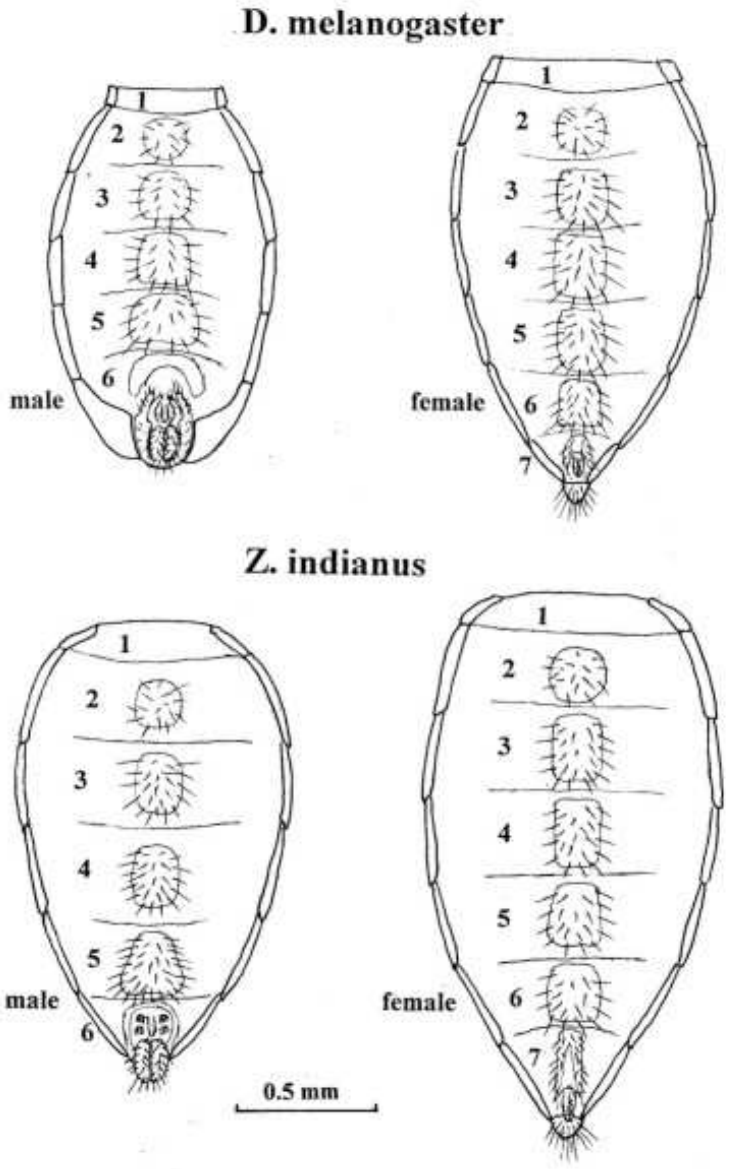

Fig. 1: Ventral view of female and male abdominal sternites in the two species investigated. In female, six sternites (A2 to A7) harbor neurosensory bristles; in males only four (A2 to A5). Notice that in *D. melanogaster* male the A6 sternite is clearly visible, but deprived of any bristle. In *Z. indianus* male, the 6th sternite is not visible.

3. Results

Basic statistics on mean bristle number

Mean values for each segment are given in Table 1. Differences between sexes are, except for segment 2 in *Z. indianus*, all highly significant, but in opposite direction according to species. More precisely, females have more bristles than males in *D. melanogaster*, while the reverse is true in *Z. indianus*. This phenomenon clearly appears when calculating the total bristle number for segments A2 to A5 (Table 1 and Fig. 2). In females, the total bristle number is close to 80 and is not significantly different between the two species. The difference is however striking between males: 66 bristles in *D. melanogaster* and 99 in *Z. indianus* (Table 1).

Variations among successive segments

Since abdominal segments share the same basic architecture, antero-posterior variations may correspond to a developmental gradient. In this respect, bristle number variation is akin to a reaction norm, and we further explore the shape of these curves, for A2 to A7 in females and A2 to A5 in males (Fig. 3).

For each case, ANOVA evidenced a highly significant line effect, corresponding to a genetic heterogeneity. The interaction line x segment was significant only in *Z. indianus* females (Table 2).

In *D. melanogaster* females, the overall shape is bowed downward, with extreme values for A2 and A7 significantly lower than those observed for intermediate segments (A3-A5). Variations among segments account for 56% of the total variability. In *D. melanogaster* males, variations among segments explained 53% of the total variability, and the curves are parallel to those of females (Table 2 and Fig. 3).

Table 1: Mean number of bristles on abdomen sternites 2 to 7 (A2-A7), for each sex and species. Comparisons among segments from the same sex are made with ANOVA, and results of post-hoc Tuckey tests are indicated (different letters indicate significant difference). Comparisons between sexes are made with Student's paired t-test. N = 12 lines in each case.

	D. melanogaster			*Z. indianus*		
Segment	*female*	*male*	*comparison*	*female*	*male*	*comparison*
A2	15.6 ± 0.26 [a]	12.6 ± 0.30 [a]	9.36***	16.7 ± 0.30 [a]	16.6 ± 0.26 [a]	0.29 [ns]
A3	21.2 ± 0.31 [b,c]	17.4 ± 0.33 [b]	20.86***	22.8 ± 0.33 [b]	24.2 ± 0.31 [b]	-3.51**
A4	21.3 ± 0.38 [b,c]	17.7 ± 0.28 [b]	10.37***	22.2 ± 0.36 [b,d]	24.6 ± 0.35 [b]	-5.92***
A5	21.5 ± 0.33 [b]	18.1 ± 0.31 [b]	11.40***	19.7 ± 0.45 [c]	34.1 ± 0.51 [c]	-22.10***
A6	19.9 ± 0.33 [c]	0		18.1 ± 0.38 [a]	-	
A7	10.0 ± 0.36 [d]	-		20.9 ± 0.46 [c,d]	-	
Sum A2-A5	79.54 ± 1.13	65.73 ± 1.11	16.24***	81.38 ± 1.25	99.39 ± 1.28	-12.65***

*Level of significance: ns, non-significant; **, <0.01; ***, <0.001.*

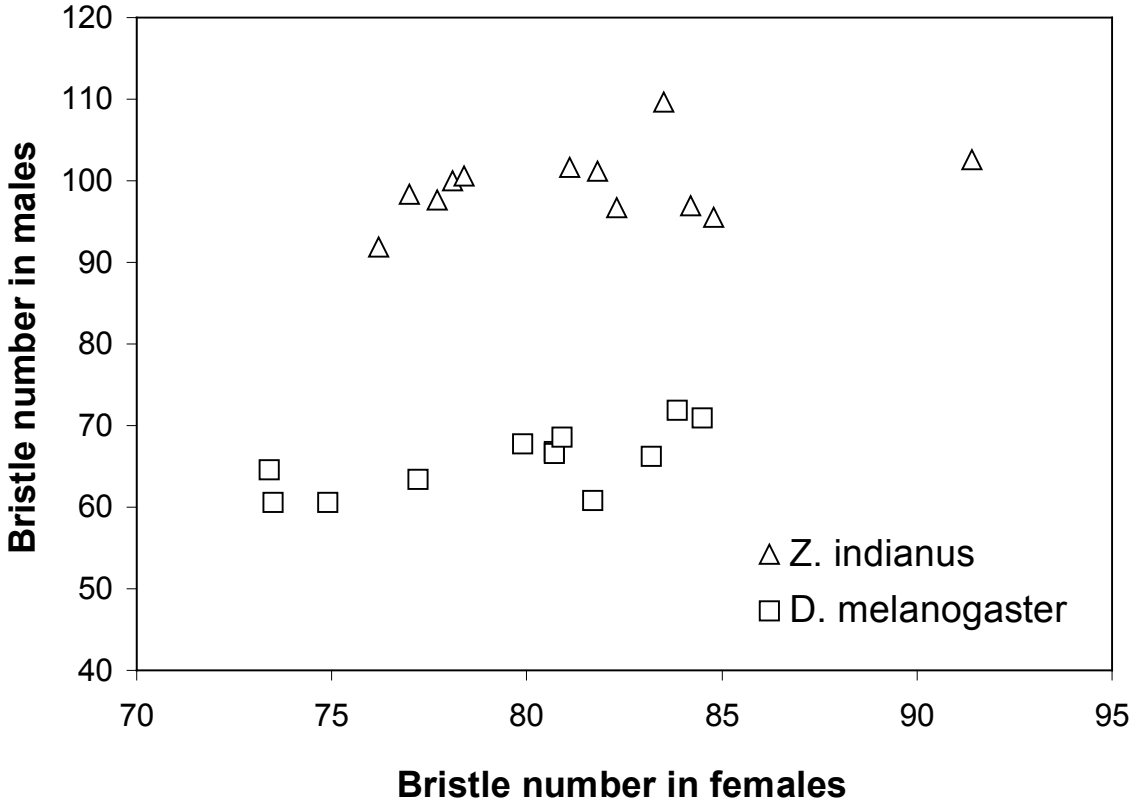

Fig. 2: Correlation diagram of the sum of abdominal bristles (A2 to A5) in females and males of the two investigated species. Notice the similarity of the females and the major difference in males. Each point is the mean of an isofemale line.

In *Z. indianus* females, variations across segments were less pronounced, accounting for only 46% of the total variability. On the other hand, in males, the between segment variability was much higher, explaining 84% of the total variation (Table 2 and Fig. 3).

Curves of second and third order were adjusted to the line-mean data of *D. melanogaster* and *Z. indianus* females, respectively (Fig. 4), with a very good fit (high correlation coefficients). This shows that, even though there is homogeneity in the number of bristles from A2 to A5 in females, the distribution pattern of these bristles in the abdomen is very different for each species.

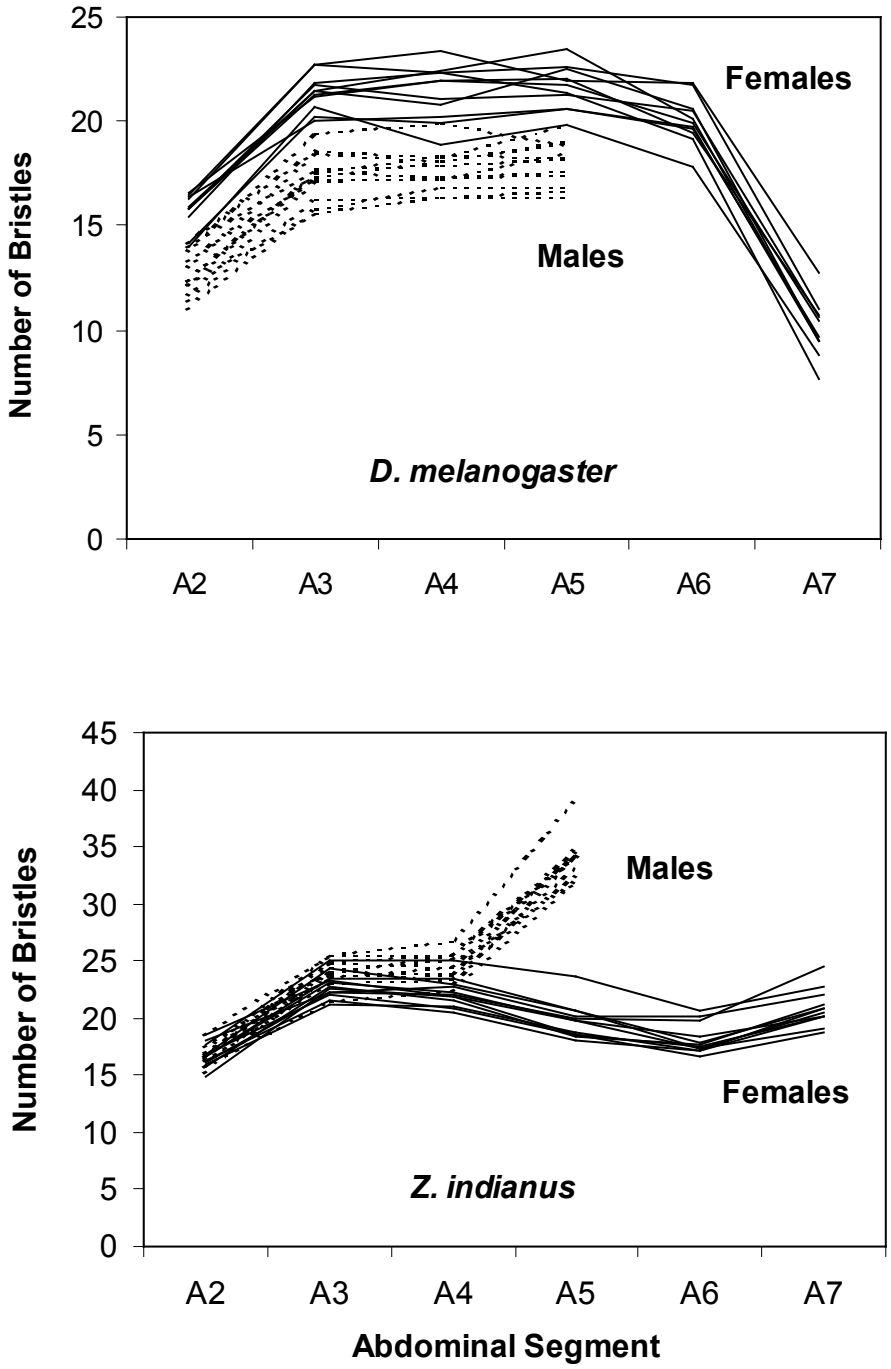

Fig. 3: Variation of bristle number in successive segments of females and males in the two species investigated. In each case the values of 12 isofemale lines are shown.

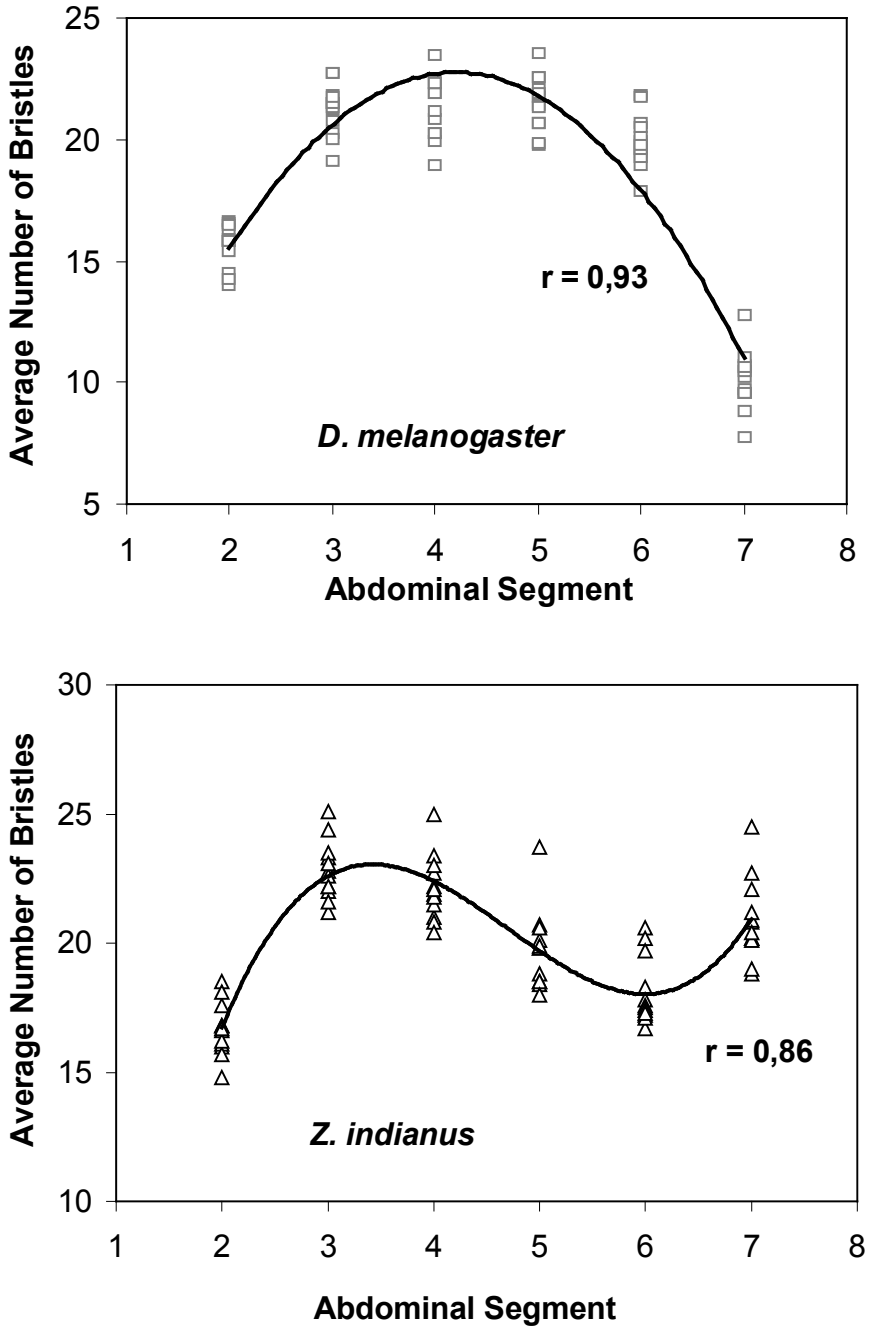

Fig. 4: Adjustment of curves of second and third order to line-mean data of females of the two species. Notice the high values for the coefficient of correlation.

Table 2: Proportion of variance explained by each component of the two-way ANOVA comparing the number of bristles among lines and segments.

		D. melanogaster		*Z. indianus*	
Effects	*df*	*female*	*male*	*female*	*male*
Lines	11	7.5***	2.4***	9.6***	9.1***
Segments	5	55.8***	53.1***	46.5***	84.0***
Lin. x Seg.	55	1.6 *ns*	0.9 *ns*	6.3***	1.8 *ns*
Residual	648	35.0	12.7	37.6	36.0

*ns: non-significant; ***, p <0.001.*

Within line variability

The isofemale line design allows calculating a variance for each line and each segment. Since mean values are variable, comparisons are easier when considering a coefficient of variation (CV). Basic data are given in Table 3 and were submitted to ANOVA (not shown).

Within sex and species, differences among segments in the CV were small and generally non-significant. The most salient feature is the very high relative variability (19.3%) for A7 in *D. melanogaster.* Differences between sexes were also non-significant, and variability was similar in the two species. If we consider the 8 values for A2-A5 in both sexes, the average is 10.08 in *D. melanogaster* and 10.09 in *Z. indianus.* For the sum A2-A5, CVs are however significantly reduced, which is due to the averaging effect on random fluctuations across segments.

Table 3: Analysis of the within-line variability. Each value is the mean CV (coefficient of variation) calculated from 12 lines. Comparisons among segments are made with ANOVA, and the same letter indicates homogeneous data (Tukey post-hoc test with $p < 0.05$). For segments A2-A5 differences among sexes are not significant and a mean value is calculated in each case. N = 12 lines in each case.

	D. melanogaster			*Z. indianus*		
Segment	*female*	*male*	*mean*	*female*	*male*	*mean*
A2	11.3 ± 0.88^{a}	12.5 ± 1.03^{a}	11.9 ± 0.65	13.1 ± 1.03^{a}	13.7 ± 0.71^{a}	13.4 ± 0.66
A3	7.9 ± 0.36^{a}	9.3 ± 0.79^{a}	8.6 ± 0.40	7.8 ± 0.60^{b}	9.1 ± 0.75^{b}	8.5 ± 0.61
A4	8.6 ± 0.88^{a}	9.8 ± 0.71^{a}	9.2 ± 0.62	8.5 ± 0.66^{b}	9.8 ± 0.97^{b}	9.1 ± 0.66
A5	10.0 ± 0.70^{a}	11.2 ± 2.24^{a}	10.6 ± 1.29	$10.1 \pm 0.58^{a,b}$	8.6 ± 0.64^{b}	9.3 ± 0.53
A6	10.9 ± 1.45^{a}	0		11.6 ± 0.98^{a}		
A7	19.3 ± 3.20^{b}	-		9.1 ± 0.81^{b}		
Sum A2-A5	5.7 ± 0.44	6.8 ± 0.56	6.2 ± 0.37	7.2 ± 0.63	7.3 ± 0.61	7.3 ± 0.43

Between line, genetic variability

Genetic variability among lines was estimated by calculating the intraclass correlation coefficient (ICC) and also the genetic CV (Table 4). There was not much variation between sexes or species. Average values of intraclass correlation for A2-A5 are: *D. melanogaster*: 0.156 $\pm$ 0.020 (n = 8), and *Z. indianus*: 0.165 $\pm$ 0.027 (n = 8). Average values of CVg are: *D. melanogaster*: 4.91 $\pm$ 0.58 (n = 8), and *Z. indianus*: 4.42 $\pm$ 0.42 (n = 8).

Table 4: ICC (isofemale line heritabilities) and evolvability (genetic CV) for each sex within species.

	ICC				*CVg*			
Segment	*mel*		*ind*		*mel*		*ind*	
	f	*m*	*f*	*m*	*f*	*m*	*f*	*m*
A2	0.11	0.21	0.08	0.04	4.01	6.56	4.06	2.71
A3	0.19	0.23	0.18	0.10	3.82	5.22	3.85	3.11
A4	0.23	0.15	0.21	0.10	4.91	4.17	4.53	3.44
A5	0.12	0.06	0.29	0.17	3.78	3.55	6.51	4.01
A6	0.09		0.18		3.69		5.62	
A7	0.17		0.30		9.43		6.34	
Total (A2-A5)	0.15	0.33	0.25	0.17	6.71	4.94	4.32	3.44

mel: D. melanogaster; ind: Z. indianus

Within line correlation

In females, since six segments harbor bristles, 15 different coefficients can be calculated, while in males this number drops to only 6. Correlation coefficients, calculated at the within line level, are given in Table 5.

Table 5: Coefficients of correlation between bristle numbers of different segments. Interval refers to the physical distance between correlated segments. Each value (± SE) is the mean of 12 lines. *f:* female; *m*: male.

Correlation	*Interval*	*Within-line*			
		D. melanogaster		*Z. indianus*	
		f	*m*	*f*	*m*
A2-A3	*1*	0.32 ± 0.09	0.09 ± 0.09	0.46 ± 0.08	0.41 ± 0.10
A2-A4	*2*	0.21 ± 0.12	0.26 ± 0.08	0.37 ± 0.13	0.32 ± 0.12
A2-A5	*3*	0.26 ± 0.12	0.15 ± 0.07	0.38 ± 0.07	0.28 ± 0.12
A2-A6	*4*	0.34 ± 0.12		0.27 ± 0.12	
A2-A7	*5*	0.40 ± 0.07		0.15 ± 0.10	
A3-A4	*1*	0.22 ± 0.15	0.29 ± 0.09	0.40 ± 0.08	0.45 ± 0.08
A3-A5	*2*	0.24 ± 0.16	0.33 ± 0.10	0.37 ± 0.10	0.35 ± 0.08
A3-A6	*3*	0.15 ± 0.12		0.20 ± 0.11	
A3-A7	*4*	0.14 ± 0.11		0.23 ± 0.08	
A4-A5	*1*	0.30 ± 0.15	0.25 ± 0.10	0.47 ± 0.09	0.29 ± 0.10
A4-A6	*2*	0.31 ± 0.12		0.39 ± 0.11	
A4-A7	*3*	0.16 ± 0.11		0.35 ± 0.06	
A5-A6	*1*	0.41 ± 0.09		0.50 ± 0.07	
A5-A7	*2*	0.35 ± 0.09		0.35 ± 0.07	
A6-A7	*1*	0.41 ± 0.08		0.48 ± 0.06	

In a modular trait we may expect that adjacent modules will be more similar than distant ones. We thus analyzed also the correlation as a function of the distance between segments. More precisely, the interval between adjacent segments is one, while the interval between the most distant segments (A2 and A7) is 5. The results for females are shown in Fig. 5 and reveal a major difference between the two species. In *Z. indianus* we notice a quite regular decrease of the correlation according to the distance between segments, from 0.46 ± 0.05 for adjacent segments

down to 0.15 ± 0.10 for the most distant. In *D. melanogaster* the correlation between adjacent segments is significantly less (0.26 ± 0.05) and decreases to 0.14 ± 0.06 for an interval of 4. For more distant segments however, we observe an increase of *r*, so that the values between A2 and A7 rise to 0.40 ± 0.07. A Mantel test showed that matrices of the two species were not significantly correlated (females: r = 0.34; males: r = 0.04). An Analysis of Covariance (ANCOVA) was applied to these data (using line as factor and the distance between segments as covariate) and revealed a significant decreasing linear component for distance in *Z indianus*. Conversely, in *D. melanogaster* there was a significant quadratic component for distance (Table 6). In both species, correlations in males were generally lower than in females.

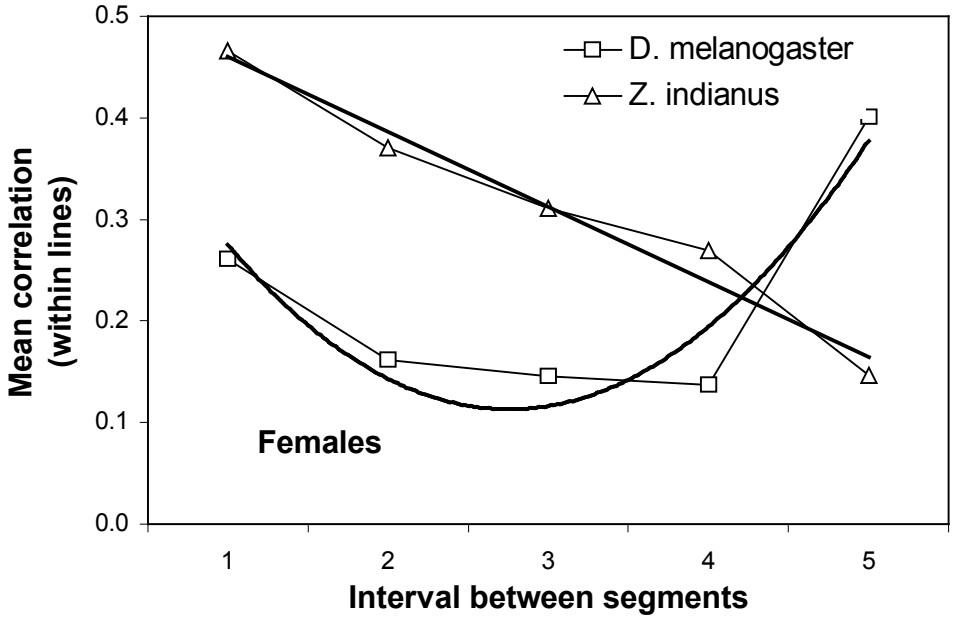

Fig. 5: Variation of the correlation between pairs of segments, as a function of the interval between segments (only female data from table 4 are shown). Data are adjusted either to a linear model of decreasing correlation according to physical distance between segments, or to a quadratic convex curve.

Table 6: Results of the ANCOVA used to test the fitting of linear and quadratic components to the curves of correlation as a function of the distance between segments.

Effects	*df*	*D. melanogaster*		*Z. indianus*	
		linear	*quadratic*	*linear*	*Quadratic*
Line	11	1.06 *ns*	0.52 *ns*	1.23 *ns*	0.71 *ns*
Distance	1	0.87 *ns*	8.13*	29.3***	0.56 *ns*
Distance2	1	--	9.62**	--	0.04 *ns*
Line x Dist.	11	0.80 *ns*	0.55 *ns*	1.63 *ns*	0.49 *ns*
Line x Dist2	11	--	0.57 *ns*	--	0.52 *ns*
Line x Dist x Dist2	11	--	0.57 *ns*	--	0.54 *ns*

*Level of significance: ns, non-significant; *, <0.05; **, <0.01; ***, <0.001.*

Sexual dimorphism

Correlations between sexes, calculated from line-mean values, are shown in Table 7. All coefficients, except one, are positive. A more detailed inspection of the two matrices in Table 7 reveals some interesting features. For example, the overall value in *D. melanogaster* is twice that found in *Z. indianus* (0.53 $\pm$ 0.08 *vs*. 0.27 $\pm$ 0.05, n = 24 in each case).

A second feature is that, in both species, A7 in females exhibits an overall low correlation: 0.25 $\pm$ 0.05 and 0.09 $\pm$ 0.10, in *D. melanogaster* and *Z. indianus* respectively. This may be related to the fact that A7 does not exist in males. However, if we consider A6, which also does not harbor bristles in males, we find that this segment in females is highly correlated with male anterior segments: 0.63 $\pm$ 0.04 in *D. melanogaster* and 0.31 $\pm$ 0.10 in *Z. indianus*. Finally, in *Z. indianus* male, A5 exhibits a very low overall correlation with female's A5 (0.09 $\pm$ 0.07), while a high correlation is observed in *D. melanogaster* (0.55 $\pm$ 0.09). This suggests that in *Z. indianus,* male A5 is specifically affected by sex dimorphism. A last observation is that correlations among

homologous segments are not better than between non-homologous segments: mean values are 0.56 + 0.10 in *D. melanogaster* and 0.35 + 0.12 in *Z. indianus* (n = 4 in each case).

The second way to analyze sexual dimorphism is to consider the F/M ratio (Table 8) and in this case only segments A2 to A5 can be considered. A two-way ANOVA showed that the ratio differs among lines. Variance ratio was $F_{11,433} = 10.30$ ($p < 0.001$) in *D. melanogaster* and $F_{11,433} = 3.09$ ($p < 0.001$) in *Z. indianus*. This significant between-line (genetic) heterogeneity explains the fairly high intraclass correlations, which are on average 0.19 ± 0.04 in *D. melanogaster* and 0.21 ± 0.05 in *Z. indianus.* The evolvability (CVg) is also high in both species, and significantly greater in *D. melanogaster* than in *Z. indianus*. ANOVA also revealed significant differences among segments (Table 8). In *D. melanogaster* these differences are small, ranging between 1.19 and 1.24, and for the sum the ratio of 1.22 means that, on average, females have 22% more bristles than males. Variations are much bigger in *Zaprionus*, and the ratio decreases regularly from 1.00 to 0.58 in A5. On average, females have less bristles than males.

4. Discussion and Conclusions

Our analysis of bristle numbers on all abdominal sternites in both sexes of two distantly related species has revealed significant differences between segments, sexes and species, as well as a number of interactions. Moreover, the use of an isofemale-line experimental design has provided some information on the genetic architecture of the various traits. For a better synthesis of the data, it seems convenient to discuss them from three points of view: the antero-posterior variations, the differences between sexes and the evolution of abdomen architecture in *Drosophila*.

Table 7: Coefficient of correlation between sexes. *F2-F7* represent abdominal segments of females and *M2-M5* the abdominal segments of males. Each coefficient is based on 12 observations.

	D. melanogaster					*Z. indianus*				
	M2	*M3*	*M4*	*M5*	*m (± SE)*	*M2*	*M3*	*M4*	*M5*	*m (± SE)*
F2	0.35	0.44	0.40	0.46	0.41 ± 0.02	0.66	0.33	0.45	0.29	0.44 ± 0.08
F3	0.85	0.83	0.72	0.81	0.80 ± 0.03	0.17	0.30	0.38	0.19	0.26 ± 0.05
F4	0.61	0.55	0.48	0.67	0.58 ± 0.04	0.07	0.45	0.35	0.06	0.23 ± 0.10
F5	0.54	0.41	0.41	0.57	0.48 ± 0.04	0.21	0.48	0.35	0.09	0.28 ± 0.08
F6	0.72	0.54	0.65	0.63	0.63 ± 0.04	0.23	0.45	0.49	0.08	0.31 ± 0.10
F7	0.38	0.16	0.25	0.19	0.25 ± 0.05	0.11	0.18	0.26	-0.20	0.09 ± 0.10
Mean (SE)	0.57 (0.08)	0.49 (0.09)	0.48 (0.07)	0.55 (0.09)	0.53 (0.08)	0.24 (0.09)	0.37 (0.05)	0.38 (0.03)	0.09 (0.07)	0.27 (0.05)

Table 8: Sexual dimorphism (F/Mm) for the number of bristles in each segment and the sum of bristles (A2 to A5). Mean and standard errors are shown. Intraclass correlation coefficients (*ICC*) and evolvability (*CV*g) are also shown.

	mean		*ICC*		*CVg*	
Segment	*melanogaster*	*indianus*	*mel*	*ind*	*mel*	*ind*
A2	1.24 ± 0.03^{a}	1.00 ± 0.01^{a}	0.26	0.04	6.99	2.63
A3	$1.22 \pm 0.01^{a,b}$	0.95 ± 0.02^{b}	0.08	0.23	2.36	4.53
A4	$1.21 \pm 0.02^{a,b}$	0.90 ± 0.02^{c}	0.21	0.24	4.73	4.85
A5	1.19 ± 0.02^{b}	0.58 ± 0.01^{d}	0.13	0.31	3.89	7.38
Total (A2-A5)	1.22 ± 0.01	0.82 ± 0.01	0.26	0.25	3.53	4.45

N = 12 lines; $^{a, b, c, d}$ indicate significant difference among segments (Tukey post-hoc test with $p < 0.05$).

Antero-posterior variations

We found in many cases significant differences among segments and curves describing these variations are shown in Fig. 2. In *D. melanogaster* females, the overall shape is concave, with a maximum number in intermediate segments. In *Z. indianus* female, the bristle numbers are quite similar to those found in *D. melanogaster*, in spite of its larger body size (Karan et al. 2000). A major difference is however observed on A7: on average 20.9 bristles in *Z. indianus,* but only 10.0 in *D. melanogaster*. In *D. melanogaster* males, variations in segments A2 to A5 are almost parallel to those of females, but with smaller values. In *Z. indianus,* on the other hand, the number increases almost linearly from A2 to A5 and becomes much higher than in females.

Besides the mean, the variance is an interesting biometrical parameter, and to get rid of mean variations, a standardized coefficient (CV) is preferred. Within lines, CVs are not very

different between segments and species (Table 3), with an overall value close to 10. In this respect, the variability of abdominal bristles, or the level of developmental instability, is similar to that found in another meristic trait, the sternopleural bristles (Capy et al. 1994; Moreteau et al. 2003). There is, however, one conspicuous exception: A7 in *D. melanogaster* female showed a CV of 20. At this level, developmental canalization seems far less efficient, although the same phenomenon does not appear in *Z. indianus*.

Genetic variations between lines can be appreciated by the coefficient of intraclass correlation (ICC), which is akin to a broad sense heritability (Hoffmann & Parsons, 1988; David et al. 2005). Overall values were quite similar in the two species (0.16 on average). An interspecific variation appears mostly for the posterior segments of both males and females. The values are slightly less than the average (0.29 ± 0.02) found in numerous natural populations of *D. melanogaster* (Capy et al. 1994). Evolvabilities (genetic CV) are also similar in both species, on average 4.7, much less than the within-line, individual variability.

Correlations between segments provided a large amount of original observations. Within lines, the correlations are always positive, indicating a common developmental component, but the values are always fairly small, lower than 0.4 in *D. melanogaster* and 0.5 in *Z. indianus*. In *Z. indianus* females, we notice a regular, linear decrease according to the distance between correlated segments (Fig. 5), from 0.46 when adjacent segments are compared, down to 0.15 for the correlation A2-A7. This result could be expected considering the existence of an antero-posterior gradient for gene expression. This may be achieved by the action of a morphogen, which emanates from a localized source and diffuses away to make a concentration gradient that promotes the regulation of genes, leading to the formation of the pattern (Turing, 1952; Lawrence, 1992; Lawrence & Struhl, 1996). The interaction of genes and morphogens occur at three levels, which act independently but at the same time overlap, leading to a very complex

genetic background for the development of the phenotype: at the segment level, at the compartment (within-segment) level and at the position level within the compartment (Lawrence & Struhl, 1996; Lawrence et al., 1999). Such a complexity in the chain of genetic interactions may generate other kinds of patterns, including the convex curve observed for *D. melanogaster*, with a maximum (0.45) between A2 and A7, which is totally different from the linear pattern of *Z. indianus*.

The sexual dimorphism

The architecture of the posterior abdomen is strongly affected by the development of the reproductive apparatus and the genitalia. In males, only six tergites are well developed, while in females seven tergites can be recognized. Sex-specific genes involved in development also affect the architecture of the ventral part of the abdomen, and the production of sternital bristles. We already know (Fig. 1) that sternite 6 is well developed in *D. melanogaster* males, while the formation of mechanosensory bristles is inhibited. In *Z. indianus*, the inhibitory effect seems stronger, since sternite 6 is not visible. Our work tried to extend the knowledge of these sex-specific effects on sternite 6 to more anterior segments, A2 to A5.

The case of *D. melanogaster* seems quite simple: the fact that female and male curves are basically parallel suggests no specific effect besides the well-known size difference. In other words, the inhibitory effect, expressed in sternite 6, does not extend further to sternite 5. In females, the last tergite (A7) exhibits some specific features. One is the bifid shape, around the ovipositor; the other is a very low bristle number and a high phenotypic variability. A quite different pattern is seen in *Z. indianus*. Considering the complete repression of sternite 6 in males, we could expect this inhibitory effect to extend to more anterior segments. However, we found exactly the reverse: A5 in males harbors almost twice the bristle number than in females,

and this excess, which is clearly evidenced by calculating the female/male ratio, disappears completely only in segment A2.

By expressing the sexual dimorphism as a ratio we were able to estimate the heritability. For body size in *D. melanogaster* (wing and thorax length), it was previously found that a much lower heritability is present for sexual dimorphism than for the traits themselves (David et al. 2003). Such is not the case for abdominal bristle number, since the ICC values (Table 7) for sex dimorphism are at least as great as those found for bristle numbers: 0.17 ± 0.04 (n = 4) vs. 0.16 ± 0.02 (n = 8) in *D. melanogaster*; 0.20 ± 0.06 (n = 4) vs. 0.15 ± 0.03 (n = 8) in *Z. indianus*.

Sexual dimorphism can be also analyzed by considering the genetic correlation between sexes. A simple analysis would be to take into account only homologous segments in both sexes. However, interesting and unexpected results were obtained by considering all pair wise combinations (Table 6). Correlations were positive, as expected, but their magnitude was similar either for comparisons between homologous and between non-homologous segments. For instance, in *D. melanogaster*, correlation between F5 and M5 is 0.57, but correlation between F2 and M5 is 0.46, and between F5 and M2 is 0.54. Indeed, the bristle number on F6, a sternite not expressed in males, is highly correlated with all male segments (0.63 ± 0.04, n = 4). A possible interpretation is that sexual dimorphism, which is genetically variable among lines, implies a set of genes that are expressed, exactly in the same way, in all segments of both sexes.

There are, however, two exceptions to this general background effect. First, bristle number on F7 exhibits either a low correlation (r = 0.25 ± 0.05 in *D. melanogaster*) or an absence of correlation (r = 0.09 ± 0.10 in *Z. indianus*) with male values. Second, bristle number on M5 is not correlated (r = 0.09 ± 0.07) with female values in *Z. indianus*. Such lacks of correlation suggest a complex determination of the phenotype for these segments. Perhaps, there are specific genes acting in F7 and in M5 in *Z. indianus*, which is characterized by a spectacular increase of bristle

number and a very low female/male ratio. Recent investigations in *Drosophila* point out for different quantitative trait loci (QTL) explaining the variation in the posterior segments of males and females (Long et al., 1995; Gurganus et al., 1998; 1999; Nuzhdin et al., 1999; Dilda & Mackay, 2002), to which our results concur.

Results for evolvability show a similar phenomenon. This suggests that sexual dimorphism for abdominal bristle number might respond more strongly to selection than bristle number itself to directional selection.

Evolution of abdomen genetic architecture

Genetic variability, both at the intra and interspecific levels, of abdominal traits in *Drosophila,* may be investigated either at the dorsal level (tergite pigmentation) or the ventral level (abdominal bristles).

The origin of segments is quite well known and related to a differential activity of homeotic genes. This modular differentiation is strongly modified by sex-specific genes, which determine the male and female genitalia, in such a way that only six segments are well developed in male and seven in female. Secondary sexual characters may also be expressed towards the end of abdomen, the most conspicuous being the black pigmentation of tergites 5 and 6 in *D. melanogaster* males (Kopp et al. 2000), and the bifid shape of sternite 7 in all females. Most of the information about the genetic background for the antero-posterior variation comes from mutational studies. These studies have revealed that both male-specific pigmentation and bristle and trichome formation are controlled by the *bab* gene, which is itself regulated by two other genes: *dsx* and *Abd-B* (Kopp et al., 2000). In males of *D. melanogaster, bab* is repressed by *AbdB* in posterior abdominal segments, allowing dark pigmentation to develop while suppressing bristles and trichomes. Conversely, high levels (equal to the female levels) of *bab* expression are

found in male segments A5 and A6 of species that do not show male-specific pigmentation, for example *D. willistoni* (Kopp et al., 2000). However, a more recent study did not find any correlation between pigmentation and bristle traits in *D. melanogaster,* and these characters seem to be controlled by different sets of QTL (Kopp et al., 2003).

Our biometrical investigations of the ventral structures have revealed many more interactions between sex and bristle number, and also different responses in the two species. In females, segment 7 exhibits a specific shape (two lobes around the ovipositor), but while the number of bristles remains high in *Z. indianus,* it is reduced and accompanied by a high variability in *D. melanogaster*. Moreover, the bristle number on this segment is poorly correlated with numbers in males of the same isofemale lines. In males, the differentiation of the posterior part of the abdomen decreases the size of A6 and prevents the development of bristles. A general conclusion is that the development of ventral structures is affected by sex but is not constrained among species.

Future investigations should be done in at least three complementary ways, which are already in course by our group: first, the comparison of more numerous species in relation with phylogeny; second, the analysis of phenotypic plasticity of abdominal bristle number, especially in relation to growth temperature; third, an analysis of genetic correlations between dorsal and ventral traits (e.g; pigmentation and bristle number) in a species like *D. melanogaster*, where such an analysis is possible.

Acknowledgements

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3. Diferenciação entre Populações

Size and shape variation within and among Brazilian populations of *Zaprionus indianus* (Diptera: Drosophilidae).

L.O. Araripe, L.B. Klaczko*

Departamento de Genética e Evolução, Instituto de Biologia, Universidade Estadual de Campinas, Unicamp, CP: 6109, 13083-9790 Campinas, SP, Brasil.

Abstract

The invasion of *Zaprionus indianus* in Brazil occurred very recently and so creates an optimal situation for the study of adaptive evolution. The history of this invasion is unknown, but its success appears in the rapid spread and broad distribution of populations of *Z. indianus* after roughly five years. We studied the amount of variation in size and shape of the wing present within and among ten populations distributed across the east of Brazil. We used wild-caught females and their laboratory-reared daughters to allow the heritability of size and shape to be estimated. The method of geometric morphometrics was used in order to visualize the regions of the wing accounting for the existing variation. We found highly significant differences for size and shape, but the among-population relationships were different in nature and laboratory. We did not find additive genetic variation for size. However, different components of shape variation, including affine and localized changes, showed significant heritability in different populations. The current work is important in the sense that it characterizes the initial state of a biological invasion in terms of the presence and organization of variability.

1. Introduction

The species *Zaprionus indianus* is a tropical drosophilid native from the African region, and it has been considered as a semi-cosmopolitan species for its rapid colonization of broad areas in the Oriental and Neotropical zones. Although restricted to warm tropical or subtropical climates (Karan et al., 1999b; Araripe et al., 2004), *Z. indianus* shows habitat-generalist characteristics and is able to adapt to variable climatic conditions (Parkash & Yadav, 1993). In the Indian subcontinent the colonization has probably occurred some centuries ago and recent studies have reported the occurrence of genetic and morphological clines according to latitude (Karan et al., 2000), which gives an evidence of natural selection and local adaptation. The invasion of *Z. indianus* in Brazil occurred very recently (Vilela, 1999; Goñi et al., 2001; Tidon et al., 2003) and has been successful in the sense that populations of this species are spread across most of the territory, as well as other regions in South America. This situation offers a good opportunity to study the process of colonization and differentiation among populations. Supposing the invasion was a unique event, one could argue that genetic divergence among populations would increase with time, and phenotypic differentiation could arise as a result, and therefore would take longer to occur.

Taking apart the effect of genetic drift as a source of variation, morphological differences among populations in nature may arise from the action of natural selection, as well as immediate non-genetic responses to the environment. An interesting approach for the study of quantitative characters is to separate these two sources of variation in order to investigate the capacity of a population to evolve. An essential condition for the occurrence of adaptive evolution in natural populations is the presence of additive genetic variance. One way of estimating the amount of additive genetic variance within a population is to look up the phenotypic covariance between relatives, or in a more practical manner, to estimate the heritability by the regression of a certain

phenotypic character between relatives (Falconer & Mackay, 1996). The degree of resemblance shown by the regression indicates the amount of genetic variation that is present in the population and thereby gives an idea of the potential for evolutionary change of the character and the rate at which it will respond to selection (Falconer & Mackay, 1996).

Since the heritability is a property not only of the trait, but also of the factors affecting a population, estimates for one population cannot be extended to others. Likewise, field and laboratory estimates for the same population are not expected to be the same. The heritability is given by a proportion of the additive genetic variance (V_A) to the phenotypic variance (V_P), so one would expect lower heritabilities in natural conditions, where more phenotypic variance is present (Riska et al., 1989; Weigensberg & Roff, 1996). However, direct comparison of field and laboratory values among several species has shown no statistical difference (Weigensberg & Roff, 1996). Attempts to obtain an approximation of the natural heritability were done by regressing the measures of wild-caught individuals with those of their laboratory-reared offspring (Coyne & Beecham, 1987; Riska et al., 1989). This allows the lower bound of "natural heritabilities" to be calculated in the laboratory.

In the present paper we study the variation in size and shape of the wing among Brazilian populations of *Z. indianus,* and access the amount of genetic variation behind these traits by estimating the heritability within and among populations. By comparing individuals from the field we expect to have an idea of the degree of divergence acquired by each population since the invasion. By rearing descendents of these individuals in laboratory conditions we expect to eliminate or reduce the differences due to environmental factors, as well as to estimate the heritability of size and shape of the wing by regressing laboratory-reared females by their wild-caught mothers.

We found that size and shape were highly variable among populations, both in nature and laboratory conditions, although a geographical or climatic pattern is not noticeable. Additionally, there is enough genetic variation within and among populations to allow adaptive evolution, which may generate important questions for future researches to be based on.

2. Material and Methods

Sample Collection

Individuals of *Zaprionus indianus* were collected in several localities of Brazil at varied times, and brought to the laboratory at Universidade Estadual de Campinas (Campinas, São Paulo, Brazil). Wild-caught females were individually placed in glass vials with regular corn medium to lay their likely inseminated eggs. The experiment was run under 24^{o}C and 12 hours/day of light. Localities and times of collections, as well as the number of isofemale lines, are shown in Table 1. In Fig. 1 the localities are graphically represented on the map of Brazil.

The females oviposited for one day and then were removed, etherized and preserved in 70% ethanol. After emergence, the offspring was aged a few days and one female from the offspring of each mother was randomly taken and preserved in 70% ethanol. Mothers and laboratory-reared daughters had their wings dissected and mounted in glass slides for morphometric analysis. We placed the wings under a Zeiss microscope and took digital pictures using the same magnification for all specimens. The digital picture of a scale was also taken under the same magnitude for size calibration. Eleven landmarks were chosen in order to characterize the whole wing (Fig. 2). We used the TPSDig software (Rohlf, 1997) to obtain landmark coordinates and also a scale factor, by which the coordinates were multiplied.

Table 1: Localities and dates of collection of *Z. indianus* in Brazil. The number of isofemale lines established is also shown.

Name	*Locality (City – State*)*	*Date*	*Number of lines*
BRA	Brasília – DF	March 2001	30
BRB	Brasília – DF	April 2001	75
BRC	Brasília – DF	July 2001	104
BRD	Brasília – DF	January 2002	49
CAA	Campinas – SP	February 2001	93
CAB	Campinas – SP	June 2001	133
GOA	Goiânia – GO	February 2002	35
POA	Porto Alegre – RS	March 2002	116
REA	Recife - PE	February 2002	62
RJA	Rio de Janeiro - RJ	April 2001	59

State abbreviations: SP, São Paulo; DF, Distrito Federal; GO, Goiás; RJ, Rio de Janeiro; RS, Rio Grande do Sul; PE, Pernambuco.

Figure 1: Graphic representation of the localities where *Z. indianus* was collected for this study.

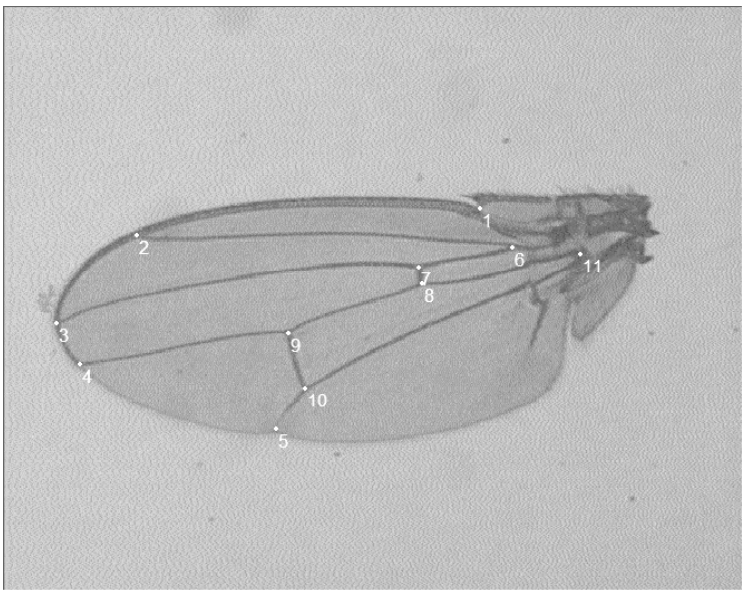

Figure 2: Right wing of *Z. indianus* showing the landmarks used for the morphometric analysis.

Size and shape variables

Landmark-based data are analyzed by methods somewhat different from the ones used in "traditional" morphometrics. First, the variables used for the analyses are the x- and y-coordinates of the positions where landmarks were digitized on each specimen. In order to be used for group comparisons these coordinates need to follow some criterions of alignment, which are 1) translating so that the centroids (configuration center of mass) are located at position (0,0); 2) scaling so that all the landmark configurations have centroid size (the square root of the summed squared distances from each landmark to the centroid) equal to one (Goodall, 1991; Rohlf, 1996); and 3) rotating to minimize the differences between each landmark and its homologous in the mean configuration. The mean configuration, also called consensus, can be estimated iteratively by a series of superimpositions. Second, size is measured by a single value that is able to represent the variation in size in all directions and is not correlated to variation in

form when allometry is absent (Bookstein, 1991). Third, it is possible to exclude the variation in size for the study and graphical reconstruction of differences in shape (Marcus, 1990; Rohlf and Marcus, 1993).

An important concept to understand the way shape is studied by the Geometric Morphometrics is the "Procrustes distance". When more than two specimens are being compared, the generalized Procrustes analysis (GPA) estimates a mean configuration and aligns the specimens to this mean as described above. Then, an individual's shape can be defined as its Procrustes distance from the consensus, i.e., the square root of the summed squared differences between each landmark and its homologous in the mean configuration (Rohlf, 1999). This metric is calculated within a spherical space, where shapes are represented by points on the superficies (Kendall, 1984; Dryden & Mardia, 1998). The projection of these points onto a tangent linear space has shown that the difference between Procrustes distances and linear distances is negligible for real data sets (for an example, see Marcus et al., 2000).

Another way to study shape is to use the partial warp scores and uniform components in multivariate analysis. Partial warp scores are obtained by projecting aligned shapes on principal warps, which derive from a between-landmark bending energy matrix that is a function of the distances between landmarks in the mean configuration (Rohlf, 1996). They describe shape variation in different scales, from the uniform global (stretching and shearing) to the localized changes (Bookstein, 1989). Because these variables are very dependent on the number and localization of landmarks on the consensus, they do not have biological significance if analyzed separately. Therefore, partial warp scores can be submitted to a relative warp analysis (RWA), which redistributes the sample variation, as well as in a regular principal component analysis. The major relative warps can then be used in regular univariate statistical analyses.

Repeatability

Estimating the repeatability of morphometric data is critical in studies of quantitative genetics because it provides a threshold value for heritability (Falconer & Mackay, 1996), and it increases the robustness of the method used to study the morphometric variation (Arnqvist & Thornhill, 1998). A sample of 30 mothers and 30 daughters were randomly taken from the population CAA. Each wing had three digital images taken, totalizing 180 images. For the centroid size and the shape parameters (aligned coordinates), the repeatability (intraclass correlation coefficient) was calculated as the proportion of: the difference between the variance among individuals and the variance within individual (i.e., between the replicates of the same individual), by the sum of these two variance components.

Size and shape variation

The experimental design we used here provides three levels of analysis: 1) comparing females from the field would reveal variation due to genetic differentiation plus environmental effects; 2) comparing females that developed all in the same laboratory conditions may reveal the amount of genetic differentiation purely, assuming they respond equally to environmental factors; 3) comparing mothers and daughters from the same population may generate an estimate of the heritability for size and shape. For the statistical analyses sample sizes were reduced to keep only the pairs (one mother and one daughter), since some females did not produce any offspring.

To compare size among groups we used the centroid size in a two-way Analysis of Variance (ANOVA) where population and family membership were used as factors. Differences between mothers and daughters within each population were investigated by paired *t*-tests.

Shape was compared in two different ways. First we tested for differences among populations by applying a multivariate analysis of variance (MANOVA) to the Procrustes

coordinates aligned to a general consensus. Significance of differences between group means was tested using Wilks' lambda. A canonical variates analysis (CVA) was conducted to visualize the relative position of the populations in the multivariate statistical space. In order to quantify and summarize the differences, Mahalanobis distances were calculated between each pair of populations and were clustered in a phenogram using the Unweighted Pair Group Method (UPGMA) in the software NTSYS-pc. In all these steps we also analyzed mothers and daughters separately, for which the coordinates were aligned to a consensus calculated for each group.

Second, we investigated the general and localized deformations associated to the differences among populations. We generated within-population consensus configurations, whose coordinates were submitted to a relative warp analysis with *alpha* = 0 (which means that both variation in small and large scale were given the same weight). The contribution of uniform components for the description of variation was evaluated by the percentage of explained variance when including them or not in the calculation of relative-warp scores (Monteiro, 1999).

Relationship between size and shape

Whether eventual differences in shape are due to size variation is an important question when comparing groups of individuals. We tested for an allometric among-population variation in shape by using a multivariate analysis of covariance (MANCOVA). In order to investigate the effect of the allometric and non-allometric components we performed a multivariate regression of aligned coordinates on the centroid size. The multivariate regression allows a prediction of specimen shape according to a given value of the independent variable, and also computes a coefficient of determination (Monteiro, 1999). The coefficient of determination (R^2) represents the percentage of variance explained by the regression. The matrix of predicted values accounts for the allometric variation.

Size and shape heritability

Within each population, heritability (h^2) of size was estimated by using the centroid size in a simple parent-offspring regression. Since only the mother was used, we multiplied the regression coefficient by two to obtain h^2 (Falconer and Mackay, 1996).

Heritability of shape was estimated by using the scores of the first five relative warps in regular parent-offspring regressions. The first five RWs were chosen according to the Scree Plot method (Jackson, 1993), which is based on a plot of the eigenvalues against the rank order. The smallest eigenvalues represent mostly the random variation and are not considered. The relative warp analysis was done on the partial warp scores (calculated with $\alpha = 0$), including the uniform components (Rohlf, 1993). We also estimated the heritability for the two uniform components isolated, in order to check for the occurrence of genetic variation in large scale. Among-population heritabilities were calculated by regressing the total set of values obtained for daughters against the total set for mothers (Coyne & Beecham, 1987).

3. Results

Size and shape variation

The repeatability was very high (>85%) for all the variables (centroid size and aligned coordinates of the landmarks), indicating that the method used for measuring the wings is highly reliable.

Mean and standard error of centroid size in mothers and daughters of each population are shown in Fig. 3. Differences in size were significant among populations ($F_9 = 42.88$, $P < 0.001$) and between mothers and daughters within population ($F_1 = 624.66$, $P < 0.001$). The interaction

between factors was also significant (F_9 = 29.94, P < 0.001), indicating that the difference between mothers and daughters was not consistent among populations. Wings of wild-caught females are clearly smaller and more variable in size than the wings of their laboratory-reared daughters. However, the difference was not significant for CAB (results of paired *t*-tests are not shown).

The wings were superimposed by Procrustes analysis and Fig. 4 shows the total variation around each landmark after alignment of the configurations. The total sample of mothers and daughters is shown (533 individuals of each). As expected, wild-caught females have more dispersed landmarks than laboratory-reared ones, which means they have more variable shapes.

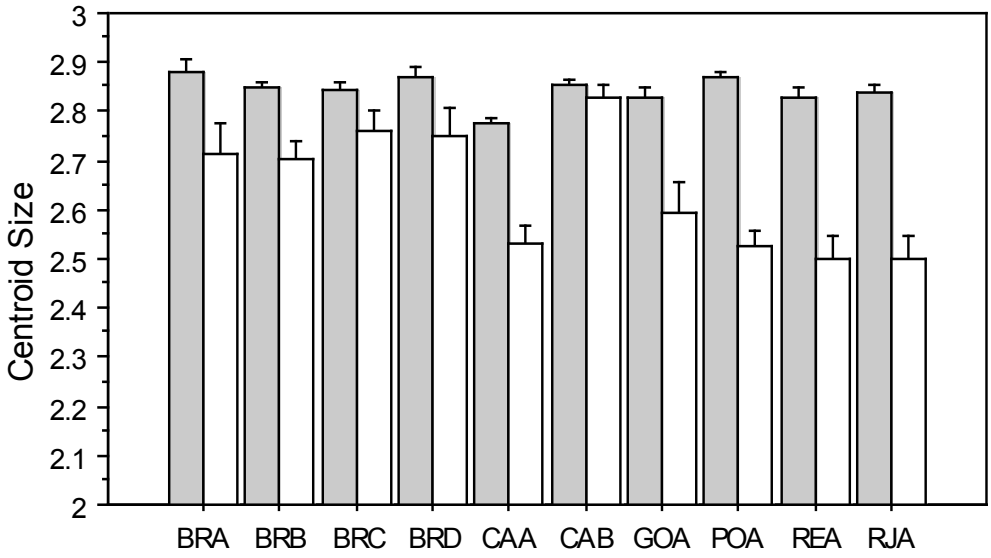

Figure 3: Mean and standard error (times two) of centroid size within each population. White bars represent the values for mothers and gray bars the values for daughters.

The MANOVA applied to compare the Procrustes coordinates among populations was highly significant for the total sample (Wilks' $\lambda = 0.413$; $F_{162,8432} = 6$; $P < 0.0001$). The result was also significant when among-population comparisons were done separately for mothers (Wilk's $\lambda = 0.237$; $F_{162,4115} = 4.941$; $P < 0.0001$) and daughters (Wilks' $\lambda = 0.381$; $F_{162,4115} = 3.213$; $P < 0.0001$). Canonical scores for the sample of mothers were plotted in the space defined by the first two canonical axes, which described 57.27% (CAN 1) and 16.30% (CAN 2) of the variation (Fig. 5A). Elipses show 95% confidence interval (Von Zuben et al., 1998).

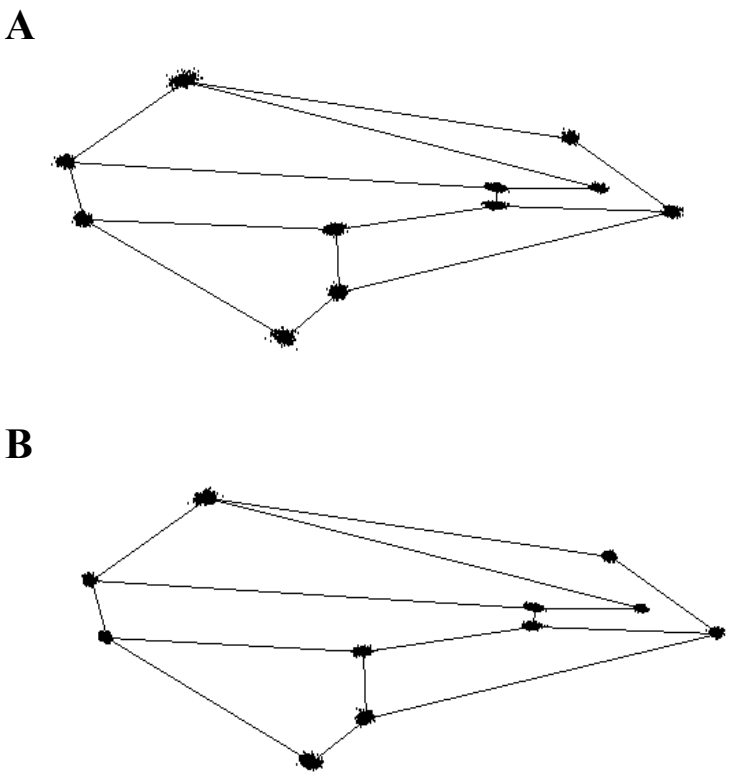

Figure 4: Procrustes superimposition of the total sample (533 mothers and 533 daughters), showing the total variation around each landmark. Lines represent links between landmarks of the consensus configuration. A) Mothers, B) Daughters.

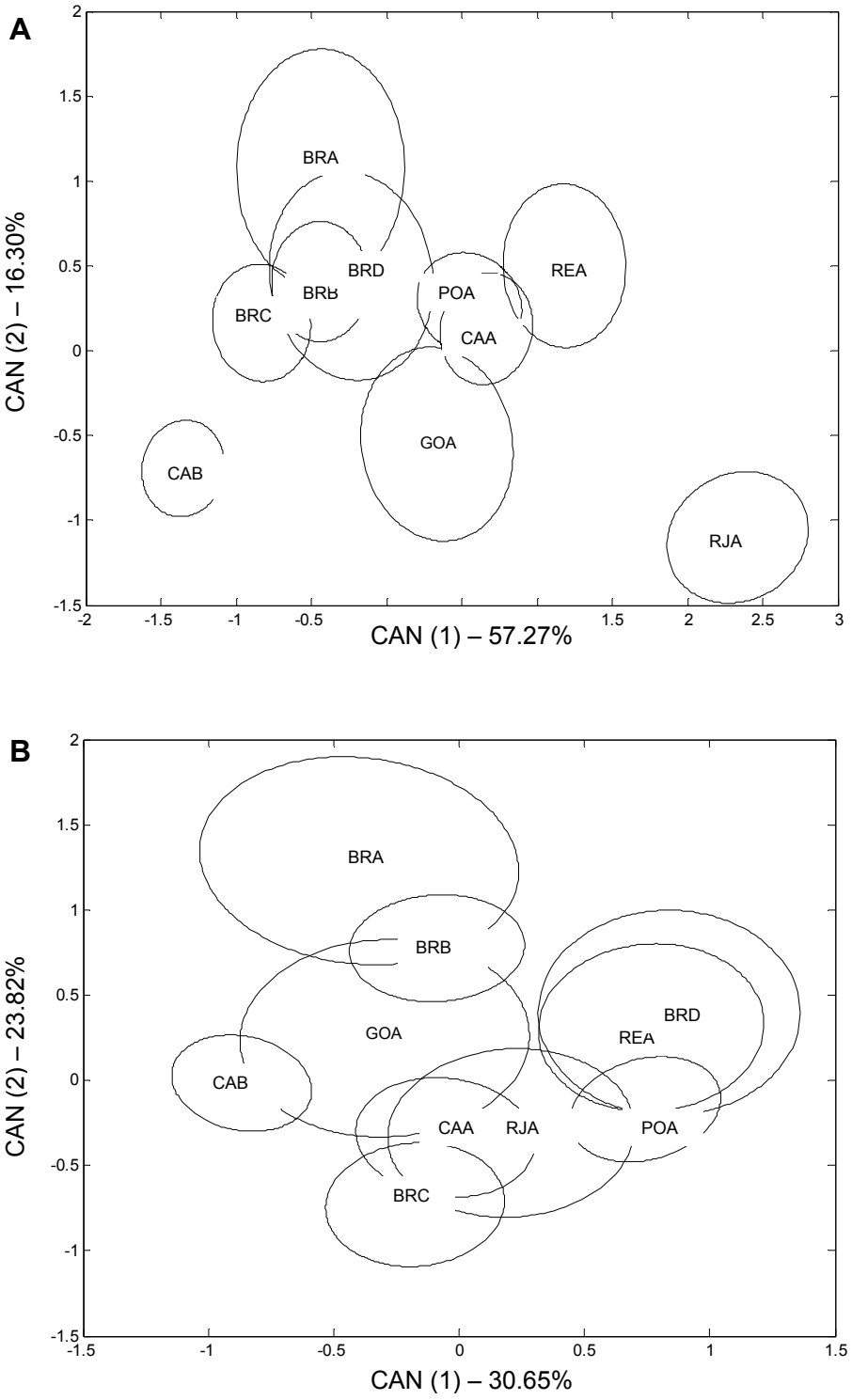

Figure 5: Ellipses comprising the 95% confidence interval of canonical scores on the plan defined by the first two canonical axes. A) Procrustes coordinates, aligned to a consensus for the sample of mothers. B) Sample of daughters.

The canonical variates analysis was also conducted for daughters separately (Fig. 5B), and for the total sample as well (not shown). The percentage of the variation in shape attributable to the first canonical axis was much lower in daughters (30.65%). For the sample of mothers we observed a good separation of populations CAB and RJA along the CAN 1 axis. Populations from Brasília (BRA-BRD) appear somehow pooled. However, populations from Campinas (CAA and CAB) are far from each other. A matrix of cophenetic values (ultrametric distances) was generated for each UPGMA tree. Only in mothers the cluster analysis was a good representation of the original matrix of dissimilarity among populations (r = 0.80) – Fig. 6. The cophenetic correlation was 0.63 in daughters and 0.54 in the total sample. One reason for the poor UPGMA representation may be the small Mahalanobis distances among populations. The matrices of Mahalanobis distances were compared between mothers and daughters using a Mantel t-test. The correlation was very small and non-significant (r = -0.01; p = 0.48).

Relationship between size and shape

The question of whether the variation in shape is related to differences in size is essential to allow a reliable interpretation of the morphometric components causing differentiation among populations. The MANCOVA shows that size effect on shape variation was significant (Wilks' λ = 0.588; $F_{18,1029}$ = 40.044; $P < 0.0001$). However, when controlling for the effect of size the differences among populations still remain highly significant (Wilks' λ = 0.718; $F_{162,8351}$ = 2.153; $P < 0.0001$). The interaction term was also significant (Wilks' λ = 0.730; $F_{162,8351}$ = 2.042; $P < 0.0001$), indicating that the allometric effect is not constant across populations. The multivariate regression of Procrustes coordinates on centroid size shows that the allometric effect is responsible for 6.88% of the variation in shape and the MANOVA on the residuals (non-

allometric component) confirms the significant difference among populations (Wilks' $\lambda = 0.449$; $F_{162,8432} = 5.409$; $P < 0.0001$).

For mothers there was also a significant non-allometric difference among populations (Wilks' $\lambda = 0.623$; $F_{162,4034} = 1.501$; $P < 0.0001$) and a significant interaction (not shown). For daughters the percent of variation explained by size is smaller (2.32%), but the allometric effect may be responsible for the significance of among-population variation in shape, so that the difference seems to disappear when we control for size (Wilks' $\lambda = 0.687$; $F_{162,4034} = 1.182$; $P = 0.061$). In fact, this result was very close to the significance and the MANOVA on the residuals of size was highly significant (Wilks' $\lambda = 0.400$; $F_{162,4115} = 3.044$; $P < 0.0001$).

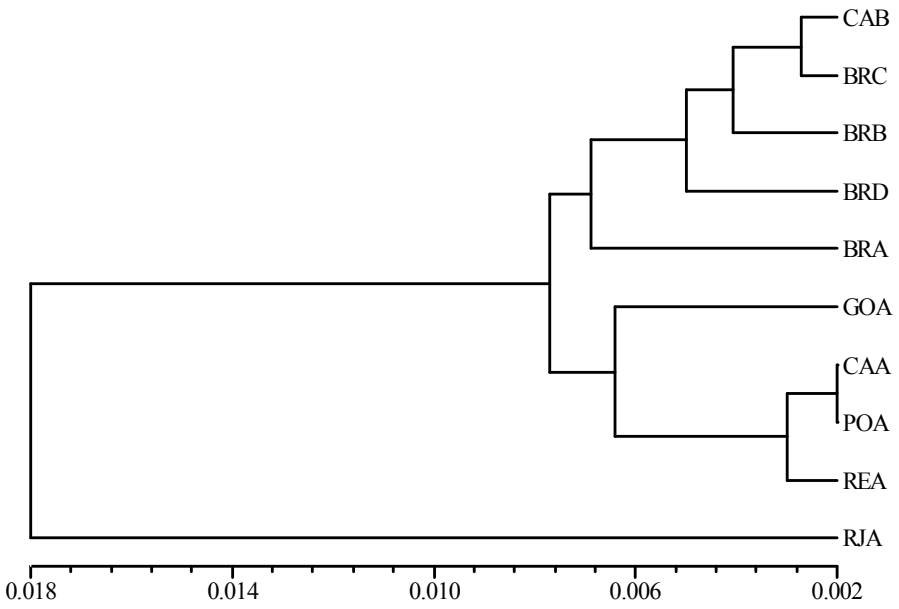

Figure 6: UPGMA phenogram derived from Mahalanobis distances calculated between pairs of populations, on the 22 aligned coordinates of the sample of mothers.

The relative contribution of uniform components to the size-based shape variation is not important: there was a difference of less than 1% in the percentage of variance explained when

the uniform components were excluded from the regression. The same occurred for the separate analysis of mothers and daughters (values not shown). Therefore we just investigated the localized size-based variation (Fig. 7).

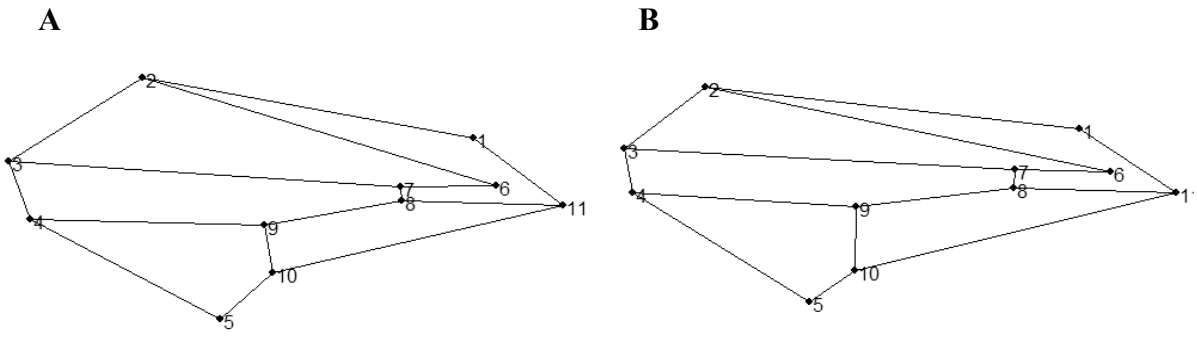

Figure 7: Variation in shape predicted by the regression of Procrustes aligned coordinates on the centroid size (does not include the uniform components). We used predicted values x 3 to make the differences more visible. A) Shape for the smallest wing; B) Shape for the largest wing.

The allometric variation in shape that is tied to an increase in size includes an approximation of landmarks #2 and #4 to landmark #3 and a displacement of landmark #10 in relation to #9, which makes the ventral part of the wing wider. The proximal wing shows a displacement of landmark #7 in relation to #8.

To visualize the deformations involved in the variation among populations, we submitted the coordinates of each-population consensus to a Relative Warp Analysis (RWA). Table 2 shows the percentage explained by the three major RWs. Figure 8 shows the deformation across the two major axes of variation (RW1 and RW2). In order to make the differences more evident relative-warp scores were multiplied by ten.

Table 2: Percentage explained for the three major axes of variation when a relative warp analysis was done on the total sample, on mothers and on daughters separately.

Source	Total sample	Mothers	Daughters
RW1	53.68	71.04	46.20
RW2	22.88	11.31	20.38
RW3	7.29	7.76	13.82

The variation described by the RW1 involves a stretching in the vertical sense and a variation in the distance between landmarks #4 e #9 (Fig. 8A and 8B). The RW2 axis describes a stretching in both senses, as well as a shearing of landmark #7 in relation to #8. The same pattern of variation across relative warp axes was observed for the sample of mothers, which might have influenced the result for the total sample. For the sample of daughters a similar pattern of variation occurred across the RW1, although the relationships among populations are somewhat different from the observed for mothers and for the total sample (not shown). The variation across the RW2 axis in daughters involves a horizontal displacement of landmark #9 and a slight shearing of landmark #7 in relation to #8.

Size and shape heritability

Centroid size did not show additive genetic variance (Table 3) in any population, which was expected for a cross-environment design. This result is indeed observed for a range of organisms in view of the plastic nature of size traits, as will be discussed later.

In most of the populations studied here we have found a significant heritability (ranging from 20% to more than 60% - Table 3) for the uniform component responsible for a stretching of the wing's general shape. On the other hand, the uniform component accounting for a shearing of the

wing exhibited lower values (ranging from 20% to 30%), which were significant in only half of the populations. The single population for which none of the uniform components have a significant genetic component is BRA. Also, in all populations but BRA, there was significant additive genetic variation for at least one of the first five relative warp axes (Table 3). In general, RW1 and RW2 were the most heritable. All shape components showed highly significant among-population heritabilities (Table 4), except the uniform component responsible for shearing. However, this one turned out to be significant when the regression was conducted on the within-population means.

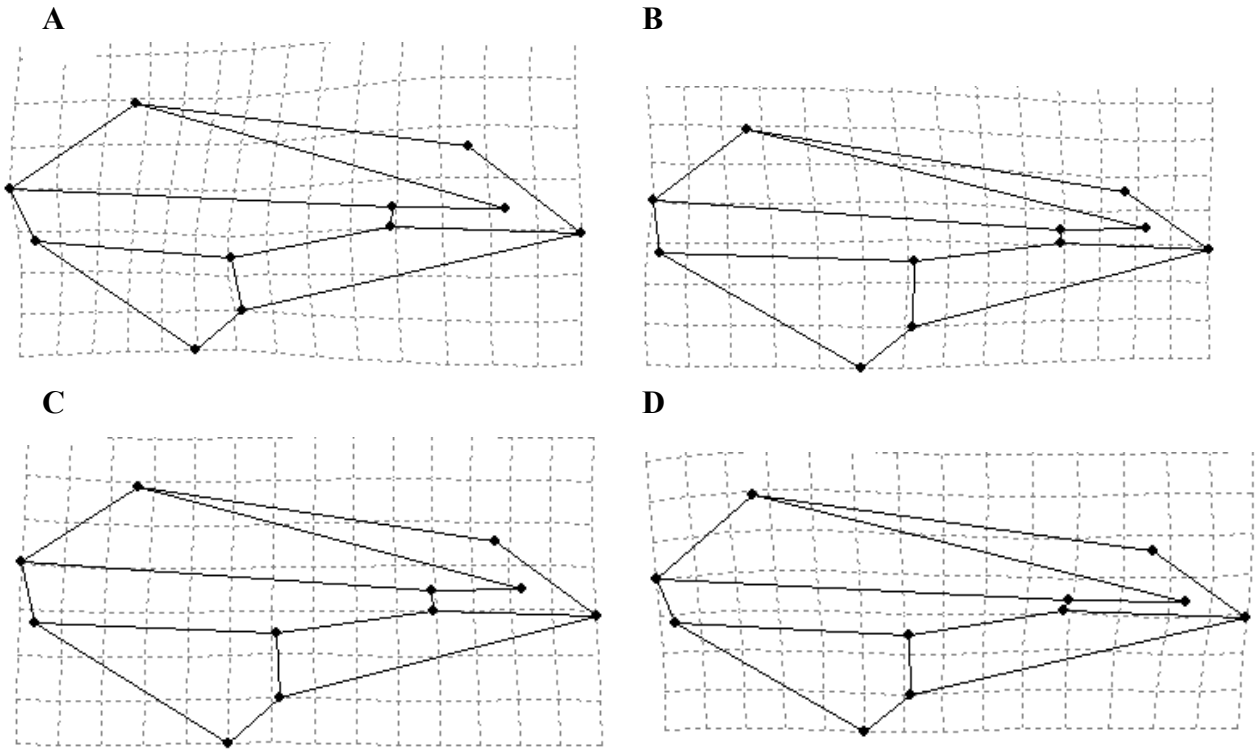

Figure 8: Deformations occurring along the relative warp axes for the variation present in the total sample. Graphs A and B show variation across the RW1 axis and C and D show variation across the RW2 axis (negative scores at the left side). RW scores were multiplied by 10.

Table 3: Estimates of heritability (h^2), calculated by parent-offspring regressions, for the centroid size and different components of shape. The standard errors are shown within parenthesis.

Origin	*N*	*Centroid size*	*U stretching*	*U shearing*	*RW1*	*RW2*	*RW3*	*RW4*	*RW5*
BRA	21	-0.212 (0.204)	-0.360 (0.338)	0.554 (0.598)	0.062 (0.474)	0.368 (0.362)	0.050 (0.258)	-0.460 (0.392)	0.378 (0.350)
BRB	60	-0.058 (0.080)	0.006 (0.266)	0.464* (0.222)	0.100 (0.170)	0.148 (0.214)	0.118 (0.220)	0.468* (0.202)	0.650* (0.266)
BRC	71	-0.038 (0.078)	0.384* (0.188)	0.582** (0.206)	0.256 (0.148)	1.022*** (0.212)	0.442* (0.188)	0.404 (0.204)	0.172 (0.200)
BRD	28	-0.218 (0.130)	1.028* (0.470)	0.566 (0.414)	0.740* (0.282)	0.410 (0.208)	0.714 (0.446)	0.268 (0.340)	0.320 (0.358)
CAA	72	0.026 (0.082)	0.536* (0.236)	0.416* (0.188)	0.484** (0.168)	0.642* (0.190)	0.212 (0.202)	0.496 (0.190)	0.276 (0.168)
CAB	96	-0.052 (0.088)	0.652*** (0.182)	0.416* (0.176)	0.656*** (0.182)	0.504* (0.208)	0.574** (0.184)	0.812*** (0.170)	0.604*** (0.162)
GOA	28	-0.172 (0.112)	0.590* (0.256)	0.324 (0.290)	0.606* (0.228)	0.664* (0.306)	0.592 (0.314)	0.618 (0.314)	0.574 (0.590)
POA	84	-0.054 (0.082)	0.282 (0.156)	0.490** (0.184)	0.336* (0.152)	0.080 (0.204)	0.200 (0.164)	0.654** (0.206)	0.660*** (0.192)
REA	37	-0.118 (0.144)	0.944** (0.266)	0.604 (0.303)	0.720** (0.240)	0.534 (0.282)	0.082 (0.294)	0.448 (0.326)	0.798*** (0.190)
RJA	37	0.084 (0.130)	0.666* (0.304)	0.318 (0.330)	0.450 (0.274)	0.660* (0.282)	0.412 (0.254)	0.688* (0.300)	0.528 (0.310)

*Level of significance: *, p <0.05;**, p <0.01; ***, p <0.001.*

Table 4: Among-population heritabilities for the components of shape, calculated either on the total set of data and on the within-population means.

Shape components	*h^2(SE) Total set*	*h^2(SE) Means*
U stretching	*-0.258 (0.086)***	*-0.778 (0.572)*
U shearing	-0.052 (0.090)	-1.508 (0.516)*
RW1	0.422 (0.064)***	0.150 (0.436)
RW2	0.370 (0.068)***	0.198 (0.228)
RW3	0.368 (0.068)***	0.512 (0.356)
RW4	0.496 (0.074)***	0.020 (0.330)
RW5	0.466 (0.074)***	1.508 (0.434)**

*Level of significance: *, p <0.05;**, p <0.01; ***, p <0.001.*

4. Discussion

The question of biological invasions is of growing interest for the field of evolutionary genetics. Vermeij (1996) classified the invasive process in three steps: arrival, establishment and integration. The last two assume that the invader species is responding to a selective regime, and moreover it is becoming part of this selective regime by its interaction with other species in the community. The arrival of *Z. indianus* in Brazil presents a rare opportunity of studying an event of invasion from its beginning. Because this is a very conspicuous species and it appeared in very low frequencies in the first collections (Vilela, 1999; Tidon et al., 2003), we believe that it was detected shortly after the invasion. The most accepted hypothesis for the introduction of this species in South America is the possible presence of adults and larvae in fruits offered to passengers of flights coming from Africa or Asia (for a review see Tidon et al., 2003). Whether

the invasion of *Z. indianus* occurred in one or more events is unknown. If a single accidental introduction occurred, one would expect to see low variability (genetic heterozygosity) due to founder effects. On the other hand, the presence of significant variation among areas could support the hypothesis of multiple events, and even the possibility that specimens are continuing to arrive. Although this dichotomy is a commonly held view, Lande (1980) showed that, even in the most extreme case of founder effect (when a single mating pair or one fertilized female found a new population), only one quarter of the heterozygosity is expected to be lost in the first generation. Furthermore, it was shown more recently, by modeling bottleneck situations, that the loss of genetic variation is not a necessary result of the decrease in population size (Carson, 1990; Whitlock et al., 1993; Cheverud & Routman, 1995; 1996). Conversely, the founding event, followed by genetic drift, drives allele frequencies towards extreme values. This produces a new genetic environment, which might be ideal for an increase in additive genetic variance when epistatic interactions among genes are present (Carson, 1990; Cheverud & Routman, 1996).

Traditional estimates of size and shape are based on distances between landmarks placed on wing-vein intersections. Some problems about the analysis of measurements based on distances (principal component analysis – PCA) can be pointed out: 1) the variation in size cannot be completely separated from the variation in shape; 2) the mathematical requirement of orthogonal axes imposed by the PCA removes the variation in shape present in the first principal component from the subsequent components; 3) variation in size may be represented by different measures in different groups (Tissot, 1988; Zelditch et al., 2004). In 1990, Klaczko and Bitner-Mathé proposed the fitting of an ellipse to the outline of the *Drosophila* wing as a method to describe size and shape. This method circumvents the major problems imposed by the traditional morphometrics: size can be estimated independently and shape can be studied by the ratio of the minor to the major axis of the ellipse. Also, the landmarks on the wing contour can be located

using the angular coordinates in a polar system, so all the measurements obtained are geometrically independent. The major advantages of the geometric morphometrics used here over the ellipse method are its generality and the possibility it offers to resume the multivariate shape in a unique measure, the Procrustes distance. However, this is a relative measure based on the sum of squares, which means that it is non-directional and may generate confusing results (see Klingenberg, 2003; but also Monteiro et al., 2002). Therefore, we only used the Procrustes distance in an exploratory way.

Our results show that the variation in size observed among populations is very significant, mostly when wild-caught females are compared (Fig. 2). The significant difference observed between mothers and laboratory-reared daughters was evident not only for the mean of centroid size, but also for the variance: wild-caught females are about twice more variable than their daughters. Since conditions in the laboratory are much more stable than in the field this result was expected and was also found in previous studies (Coyne & Beecham, 1987; Gibert et al., 1998; Bitner-Mathé & Klaczko, 1999). The great phenotypic variation promoted by variable field conditions is considered as a reason for estimates of size heritability in nature being generally low and non-significant (Prout & Barker, 1989; Gibert et al., 1998; Bitner-Mathé & Klaczko, 1999a, b). Another reason may be that cross-environment estimates are biased by the possible occurrence of a genotype-environment interaction (Falconer & Mackay, 1996). However, we do not observe the same phenomenon for shape heritability (Bitner-Mathé & Klaczko, 1999a, b; Gilchrist & Partridge, 2001; Matta & Bitner-Mathé, 2004), as will be discussed later.

Among-population variation measured on the Procrustes aligned coordinates was very significant, although the pattern of differentiation revealed by plotting the first two canonical axes shows some overlapping (Fig. 5). The greatest overlapping occurred when daughters were used separately (Fig. 5B), indicating that part of the variation, due to immediate response to

environmental conditions, was suppressed when individuals were reared in the laboratory. The UPGMA based on dissimilarity data (Mahalanobis distances) produced an apparently confusing clustering of the populations studied here (Fig. 6), but because the invasion of *Z. indianus* in Brazil occurred very recently, we did not expect that a geographical or climatic pattern would appear from our data. In fact, we found that even populations collected at the same locality were sometimes not clustered together (e.g. CAA and CAB). Moreover, a Mantel test comparing the matrix of Mahalanobis distances with linear geographical distances between pairs of localities did not show a significant correlation.

Some of the relationships obtained can be better understood if the times of collection are considered. The grouping of CAB and BRC has probably occurred because both were collected during the winter. The same could explain the grouping of GOA, CAA, POA and REA, which were collected during the high summer, though in different years. This result may be taken as an indication that at least some components of shape can respond rapidly to climate, probably temperature changes, as already reported by Debat et al. (2003). Interestingly, we found a very different clustering pattern when only laboratory-reared flies were considered (not shown). Assuming that the flies in our sample respond equally to environmental conditions, we expect differences among laboratory-reared flies to be genetically based. Thus, differences among wild populations may have a plastic component responding to external factors like seasonality, which is probably making up the genetically based variation.

The relative warp analysis on the aligned coordinates allowed us to localize the wing regions accounting for the variation observed. A general pattern of variation seems to be the contraction of the distal area of the wing, characterized by the approximation of landmarks #2 and #4 to landmark #3, which was also seen in studies using other methods to measure the wing in varied *Drosophila* species (Bitner-Mathé & Klaczko, 1999b, c; Gilchrist et al., 2000; Hoffman

& Shirriffs, 2002; Debat et al., 2003; Matta & Bitner-Mathé, 2004). This localized variation was observed along the first relative warp axis and it is likely tied with the affine component that makes the wing more elongated (Fig. 8). Further, the significant but small effect of size on shape variation also accounts for the vertical contraction of the wing, the contraction happening when size increases (Fig. 7). Therefore, smaller wings are more rounded. Another pattern of variation clearly observed along the RW1 axis and also as an allometric variation present in shape is the movement of landmark #9 internally and proximally when size and RW scores increase, which makes the sub region at the bottom of the wing relatively bigger (Figs. 7 and 8). The major changes occurring along RW2 are the approximation of landmarks #7 and #8 and the movement of landmark #2 toward the posterior region when RW scores increase, which makes the wing more elongated.

Interestingly, although allometric variation accounted for a small proportion of shape variation, its effects are very similar to the deformation observed along the first RW axis. This probably happens because the possibilities of changes in the wing shape are somehow constrained throughout development and the action of different factors would lead to similar final effects. At least a small part of this constrained variation is easy to predict if we think that a contraction of the wing's vertical axis would drive some landmarks to new positions on the outline (Matta & Bitner-Mathé, 2004). Therefore, an absolute increase in size due to an increase in the length of the wing's major axis would have the same effect on localized variation as a relative increase of the major axis that makes the wing more elongated. A phenotypic correlation between size and the wing outline has been found in some studies, although there is recent evidence that different sets of genes might be regulating these traits (Matta & Bitner-Mathé, 2004).

Shape heritability was studied here as the proportion of additive genetic variation shown by the major components of shape variation: the first five relative warps and the uniform components. We also tried to estimate the heritability of shape by using the Procrustes distance, as suggested by Monteiro et al. (2002), but we obtained non-significant results. Moreover, this method may generate doubtful results in view of the non-directional nature of data that are based on distances (Klingenberg, 2003). Additive genetic variation for wing shape has been found in different *Drosophila* species (Bitner-Mathé & Klaczko, 1999b; Gilchrist & Partridge, 2001; Hoffmann & Shirriffs, 2002; Matta & Bitner-Mathé, 2004; Moraes & Sene, 2004). The presence of a genetic component for shape seems to be a general pattern even when cross-environment estimates are used, in opposition of what is observed for size. A possible explanation for this is that size is a more plastic character and so is able to respond immediately to environmental conditions as temperature or density. Thus, the wing size of laboratory-reared offspring would be somehow untied to the size and the conditions experienced by their wild-caught parents.

Although our method has probably underestimated heritability because of the environmental differences in which mothers and daughters have developed, we still found high and significant values for shape components. This suggests that Brazilian populations of *Z. indianus* have a substantial potential for evolutionary change on these traits. Because shape is a multivariate character it is hard to predict what the responses to selection would be, unless by part of the localized changes that are constrained by the variation in size and affine components. An important genetic component was also found for the among-population variation in nature, as shown by the highly significant estimates of among-population heritability.

The finding of high genetic variation within and among populations could be considered a paradox given that the invasive population was probably submitted to a bottleneck. However, as pointed out before, a very small portion of the original variability is lost in the founding event,

even when only one fertilized female is the founder of the new population (Lande, 1980). Moreover, the founding event may promote the rising of additive genetic variance when a system of interacting genes is present, which must be the case for polygenic traits like size and shape (Carson, 1990; Whitlock et al., 1993; Cheverud & Routman, 1995; 1996). Alternatively, the loss of genetic variation after a bottleneck could be compensated by the addition of new variation, leading the population to a successful colonization and adaptive evolution. It was recently suggested that a successful invasion is linked to the occurrence of multiple introductions, by which new genetic variation is added into introduced areas (Kolar & Lodge, 2001; Kolbe et al., 2004). The history of invasion of *Z. indianus* in Brazil is unknown. The significant amount of genetic and morphological variation found here could support the hypothesis of multiple invasions. However, our estimates are accompanied by high error values, and we cannot reject the hypothesis of a unique event of invasion: recent investigations on the allozime diversity (Machado et al. in press) and the frequency of chromosomal inversions (Ananina, personal communication) indicate that Brazilian populations are still very uniform in the genetic composition.

Nevertheless, the goal of our study was to report the initial state of morphological variation in Brazilian populations of *Z. indianus*. It is important that this investigation be continued in the same area so that relevant information as the time elapsed until adaptive evolution (for instance, cline formation) can be generated.

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CONCLUSÕES

A invasão e o estabelecimento da espécie *Zaprionus indianus* no Brasil ocorreram recentemente e sua eficiência deve-se provavelmente a suas características generalistas e capacidade de adaptar-se a diferentes condições ambientais (Parkash & Yadav, 1993). Tais características indicam que *Z. indianus* é potencialmente cosmopolita, mas algumas diferenças fisiológicas existem com relação a outras espécies com o mesmo *status*, como *D. simulans* e *D. melanogaster:* machos de *Z. indianus* têm uma maturidade atrasada com relação a *D. melanogaster,* além de uma menor fecundidade, o que pode estar correlacionado com um maior tamanho do espermatozóide. A tolerância térmica medida através dos limites de temperatura induzindo a esterilidade do macho, também mostra algumas diferenças com relação a *D. melanogaster.* Machos desta espécie são mais tolerantes ao frio e levam o mesmo tempo para recuperar a fertilidade tanto quando submetidos a um estresse de temperatura baixa quanto a um estresse de temperatura alta. Em *Z. indianus,* a recuperação ao estresse de temperatura baixa é mais rápida que ao de temperatura alta.

Com estes resultados podemos ter uma idéia das condições ambientais favoráveis à dispersão de *Z. indianus*, além da possibilidade de prever o impacto de um estresse de temperatura sobre uma população dessa espécie.

A rápida dispersão de *Z. indianus* no Brasil abriu questões acerca da origem da população invasora e da quantidade de variabilidade presente no momento da invasão. Como as invasões biológicas são em muitos casos acidentais, espera-se que sejam protagonizadas por poucos indivíduos. Ao mesmo tempo que isso gera perda de variabilidade genética por fixação de alelos, a variação genética aditiva pode aumentar se interações epistáticas entre os genes em questão estiverem presentes (Carson, 1990; Cheverud & Routman, 1996). Uma outra maneira de ocorrer aumento de variabilidade genética depois de um gargalo populacional é através da injeção de

nova variabilidade genética. Recentemente foi sugerido que o sucesso de um evento de invasão está ligado à ocorrência de introduções múltiplas, que seriam a fonte de nova variação genética nas áreas invadidas (Kolar & Lodge, 2001; Kolbe et al., 2004).

O estudo de populações brasileiras de *Z. indianus* revelou a ocorrência de alta variabilidade fenotípica dentro e entre populações, além de variação genética aditiva suficiente (dada pelos altos valores de herdabilidade obtidos) para promover a evolução de caracteres relacionados à forma da asa. Apesar da história da invasão não ser conhecida, os resultados aqui encontrados podem ser extrapolados para sugerir que tenha ocorrido mais de um evento de invasão. De qualquer naneira, não se pode descartar a hipótese de um único evento; pesquisas recentes acerca da diversidade de aloenzimas (Machado et al., no prelo) e frequência de inversões cromossômicas (Ananina, comunicação pessoal) indicam uniformidade genética em populações brasileiras.

Independentemente da história de invasão, este estudo é importante por caracterizar o estado inicial da variação em populações brasileiras de *Z. indianus*. A continuação dessa pesquisa nas mesmas áreas torna-se muito interessante porque pode gerar informações sobre o tempo necessário para a ocorrência de evolução adaptativa, como por exemplo, o tempo necessário para a formação de clines.

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