UNIVERSIDADE ESTADUAL DE CAMPINAS

mestrado

INSTITUTO DE BIOLOGIA

UNIVERSIDADE ESTADUAL DE CAMPINAS

Luciane Mendes Hatadani

POLIMORFISMO DE COLORAÇÃO EM *DROSOPHILA MEDIOPUNCTATA*

Dissertação apresentada ao Instituto de Biologia da Universidade Estadual de Campinas para obtenção do título de mestre em Genética e Biologia Molecular na área de Genética Animal e Evolução

Orientador: Louis Bernard Klaczko

Campinas, 2002

FICHA CATALOGRÁFICA ELABORADA PELA BIBLIOTECA DO INSTITUTO DE BIOLOGIA - UNICAMP

Hatadani, Luciane Mendes

H28e Polimorfismo de coloração em *Drosophila mediopunctata*/ Luciane Mendes Hatadani. --
Campinas, SP:[s.n.], 2002

Orientador: Louis Bernard Klaczko
Dissertação (mestrado) – Universidade Estadual de Campinas. Instituto de Biologia

1.Pigmentação. 2.Polimorfismo. 3.Seleção natural. I.Klaczko, Louis Bernard. II.Universidade Estadual de Campinas. Instituto de Biologia. III. Título.

DATA DE DEFESA: 26/11/2002

Banca Examinadora

Prof. Dr. Louis Bernard Klaczko (Orientador)

Profa. Dra. Vera Lúcia da Silva Valente Gaeiśky

Profa. Dra. Vera Nisaka Solferini

Prof . Dr. Sérgio Furtado dos Reis

Aos meus pais, Ruth e Arnaldo.

AGRADECIMENTOS

Aos meus pais, Ruth e Arnaldo, por terem priorizado sempre a educação. Se não fosse por eles, eu certamente não alcançaria a formação que tenho e nem teria o mesmo interesse pelo conhecimento. E por tudo o mais, que de tão óbvio torna-se desnecessário de ser dito.

A Izabella, minha irmã, por ter me ensinado boa parte daquilo que aprendi em estatística e estar sempre disposta a tentar resolver minhas dúvidas.

Ao Alexandre, meu irmão, por ter mantido sempre o computador funcionando e, dessa forma, evitado muitas angústias, e por ter me proporcionado todo o conforto e todo o apoio quando vim morar com ele em Campinas.

Aos dois, Izabella e Alexandre, por terem escutado durante todos estes anos minhas conversas sobre "as pintas das mosquinhas" com todo o interesse possível e pela amizade e apoio sempre.

Ao Hermes, por ter lido estes artigos inúmeras vezes com tanta atenção e por ter feito tantas sugestões, de tanto valor. Obrigada por ter estado comigo durante todo este período tão penoso que foi a redação e o processo de defesa (por tantos motivos), e por ter mantido a paciência e o carinho.

À Luciene e à Cristina, porque amigos verdadeiros são muito poucos, mas parecem eternos.

À Wilma, à D. Cida, ao seu Laércio e à Patrícia, técnicos do laboratório, cuja ajuda foi fundamental para a realização dos meus trabalhos no laboratório. Wilma, obrigada por ter feito sempre mais do que o necessário para fazer com que o laboratório funcionasse.

Aos meus colegas de laboratório, Hermes, Gália, Horácio, Roberto, Cacá, Luciana, Marcão e Felipe, pela companhia, pelas conversas, pelos cafés e pela ajuda, sempre que foi possível. E pela ajuda fundamental nas coletas em Santa Genebra.

Ao prof. Jean David e à profa. Brigitte Moreteau, pelas importantes sugestões na análise dos dados do primeiro artigo.

Ao André Victor, pela ajuda para tirar as fotos das moscas.

Aos profs. Ângelo Pires do Prado, Sérgio Furtado dos Reis e Vera Nisaka Solferini, membros da pré-banca, pelas sugestões e correções feitas.

À CAPES, pela bolsa de mestrado.

Ao Prof. Louis Bernard Klaczko, meu orientador, por ter me proporcionado, desde a iniciação científica, uma excelente formação. Pela paciência, pelo incentivo, pela confiança, pela amizade, obrigada.

"O todo sem a parte não é todo,

a parte sem o todo não é parte,

mas se a parte o faz todo, sendo parte,

não se diga, que é parte, sendo todo."

Aristóteles

RESUMO

O grupo *tripunctata* é o segundo maior grupo neotropical em número de espécies, sendo abundante em florestas. A maioria das espécies desse grupo apresenta três manchas escuras no quarto, quinto e sexto tergitos abdominais, de onde vem o nome do grupo. Entretanto, em algumas espécies este padrão é variável, como é o caso de *Drosophila mediopunctata*. Há moscas sem nenhuma mancha até moscas com três manchas. Este trabalho é dividido em duas partes principais. Na primeira parte, foram estudados os padrões de manchas abdominais de duas populações naturais de *D. mediopunctata*. Foi possível determinar a influência da temperatura e da densidade durante o desenvolvimento e concluir que a densidade em geral não afeta – ou afeta pouco – o polimorfismo e que a temperatura tem um efeito intenso, de modo que moscas criadas em temperaturas mais baixas tendem a ter mais manchas. O padrão de coloração das moscas vindas do campo apresentou variação no sentido esperado pela variação de temperatura local em ambas as localidades. Entretando, ao contrário do que ocorre em outras espécies de *Drosophila*, a variação genética ocorreu em sentido oposto ao determinado pela variação local de temperatura, sugerindo a ação de seleção natural e produzindo um padrão de variação contra-gradiente. Na segunda parte do trabalho, é apresentada uma análise genética (cromossômica), mostrando que o cromossomo II (que apresenta polimorfismo de inversões) é o que tem maior importância na determinação genética do polimorfismo. A partir da análise de estirpes em que diferentes cromossomos II foram colocados sobre o mesmo *background* genético e da prole dos cruzamentos entre elas, em condições padronizadas de temperatura e densidade, encontrou-se uma associação não-aleatória entre o número de manchas e as inversões *PA0* e *PC0*. É possível que estas inversões estejam acumulando genes adequados para cada uma dessas condições de temperatura, inclusive genes que determinam o número de manchas nos tergitos.

PALAVRAS-CHAVE: pigmentação, inversões cromossômicas, contra-gradiente, seleção natural, análise genética.

ABSTRACT

The *tripunctata* species group of *Drosophila* is the second largest Neotropical group in number of species, being abundant in forests. Most of the species of this group present three dark spots on the fourth, fifth and sixth abdominal tergites, thus the name *tripunctata*. However, in some species, this pattern is variable; such is the case of *Drosophila mediopunctata*. There are flies ranging from zero to three spots. This study is divided in two parts. In the first part, the abdominal pigmentation patterns of two natural *D. mediopunctata* populations were investigated. It was possible to determine the influence of temperature and larval density on the colour polymorphism and to verify that density, in general, has little effect on the polymorphism and that temperature has an intense effect so that : flies raised in lower temperatures tend to have more spots. The colour pattern of the flies collected from the field presented the expected variation according to the variation of the local temperatures in both sites. Contrary to what happens in other *Drosophila* species, the genetic variation occurred in the opposite direction to that determined by the environmental variation, a counter-gradient pattern, suggesting the action of natural selection. On the second part of this study, a genetic (chromosomal) analysis is presented showing that the second chromosome (which is polymorphic for inversions) is the major contributor to the genetic determination of this color polymorphism. The analysis of strains in which different second chromosomes were placed on the same genetic background and the crosses among them (raised at specified conditions of temperature and larval density) showed that there was a non-random association between the number of spots and inversions *PA0* and *PC0*. These inversions may involve the accumulation of are accumulating genes adequate for each temperature, including genes that determine the number of abdominal spots.

KEYWORDS: pigmentation, chromosome inversions, counter-gradient, natural selection, genetic analysis.

SUMÁRIO

LISTA DE FIGURAS

Temporal Variation in the Colour Polymorphism of Two Natural Populations of *Drosophila mediopunctata.*

Color polymorphism in *Drosophila mediopunctata*: genetic (chromosomal) analysis and non-random association with chromosome inversions.

LISTA DE TABELAS

Temporal Variation in the Colour Polymorphism of Two Natural Populations of *Drosophila mediopunctata*

Color polymorphism in *Drosophila mediopunctata*: genetic (chromosomal) analysis and non-random association with chromosome inversions

INTRODUÇÃO GERAL

Polimorfismos conspícuos

Ford (1964) define polimorfismo genético como "a ocorrência simultânea na mesma localidade de duas ou mais formas descontínuas de uma espécie, de tal forma que a mais rara delas não possa ser mantida apenas por mutação recorrente".

Polimorfismos conspícuos são amplamente reconhecidos como estando sob a ação da seleção natural. Mesmo Sewall Wright (1978) reconheceu que polimorfismos conspícuos são particularmente favoráveis para o estudo da seleção natural. Wright considerava que estes polimorfismos, ainda que não sejam típicos da variação da qual a evolução depende, são bastante fáceis de estudar e muitos deles constituem adaptações interessantes.

Supõe-se que a maior parte dos polimorfismos conspícuos esteja sob seleção natural. Segundo Wright (1978), pode-se dizer que qualquer polimorfismo conspícuo persistente deve estar sujeito a grandes pressões seletivas, uma vez que não poderia ser mantido apenas por mutação recorrente. Na maior parte dos casos, de acordo com o mesmo autor, o polimorfismo é mantido por um equilíbrio entre pressões seletivas opostas por meio de vantagem do heterozigoto, pela existência de ambientes heterogêneos ou por uma vantagem da diversidade em si mesma.Um dos primeiros trabalhos que demonstraram a ação da seleção natural sobre polimorfismos conspícuos foram feitos por Cain e Sheppard (1950, 1954), que estudaram os padrões de manchas nas conchas de *Cepaea nemoralis*. Desde então, o polimorfismo nesta espécie e em outras espécies do gênero tem sido intensamente estudado e a maior parte das hipóteses propostas para explicar os padrões de polimorfismo nesta espécie envolvem seleção natural (ver revisão de Jones *et al.*, 1977).

A pigmentação em Drosophila

Existem no gênero *Drosophila* cerca de 2000 espécies (Powell, 1997). Entretanto, poucos casos de variação conspícua foram estudados, entre eles *D. polymorpha, D. kikkawai, D. lebanonensis, D. melanogaster* e *D. simulans*.

A coloração do abdome em *D. polymorpha* é um dos casos mais bem estudados (Da Cunha, 1949; Heed e Blake, 1963; Martinez e Cordeiro, 1970). Esta espécie apresenta variação na coloração abdominal, havendo um grande contraste entre os indivíduos mais claros e os mais escuros. Da Cunha (1949) foi o primeiro a estudar o polimorfismo e identificou um loco e dois alelos (sem dominância) responsáveis pelos fenótipos. Mais tarde, foram encontrados um novo alelo (Heed e Blake , 1963) e modificadores que segregavam indepentemente do loco principal (Martinez e Cordeiro, 1970).

D. kikkawai (Freire-Maia, 1964, Gibert *et al.*, 1999) também apresenta polimorfismo na pigmentação do abdome, determinado por um loco com dois alelos (Gibert *et al.*, 1999). Em *D. lebanonensis*, a cor do tórax é determinada por um loco com dois alelos e existem também evidências de modificadores (Pipkin, 1962).

Finalmente, *D. melanogaster* e *D. simulans* apresentam um polimorfismo de coloração do tórax que consiste na presença ou ausência de uma mancha escura em forma de tridente. A intensidade de pigmentação do tridente também é variável. Nestas duas últimas espécies, foram encontrados clines em que, quanto maior a latitude, maior a pigmentação. Esta similaridade entre diferentes espécies constitui um forte argumento a favor do significado adaptativo do cline (Capy *et al.*, 1988).

No que diz respeito à temperatura, a pigmentação em *Drosophila* em geral apresenta plasticidade fenotípica, de modo que animais que se desenvolvem sob temperaturas mais baixas tendem a apresentar uma coloração mais intensa, enquanto aqueles que se desenvolvem sob temperaturas mais altas tendem a ter fenótipos mais claros. A explicação adaptativa que tem sido aceita é a de que fenótipos escuros são mais vantajosos em temperaturas baixas, porque aumentam a absorção de calor e, conseqüentemente, a temperatura interna do inseto (Capy *et al.*, 1988; Gibert *et al.*, 1996; Gibert *et al.*, 1999). Essa hipótese foi proposta por Watt (1968), que observou que borboletas do gênero *Colias* com asas mais escuras se aquecem mais rápido e mantêm o calor por mais tempo que as borboletas com asas claras. Watt (1969) observou também que havia variação sazonal no polimorfismo de coloração e propôs que essa sazonalidade houvesse evoluído para maximizar o aquecimento no inverno e minimizá-lo no verão. O autor não esclarece, no entanto, se esta sazonalidade se deve a fatores genéticos ou ambientais.

Várias evidências que fortalecem essa hipótese adaptativa foram encontradas em drosófilas. Em *D. melanogaster* foi encontrado um cline de pigmentação do abdome, em que quanto maior a latitude maior a intensidade de pigmentação (Das, 1995). O mesmo ocorre para a coloração tridente de *D. melanogaster* e *D. simulans* (Capy *et al.*, 1988). Para a pigmentação do abdome, estas duas espécies também apresentam evidências de que populações que vivem em locais de clima mais frio são geneticamente mais escuras (Gibert *et al.*, 1996). Já em *D. kikkawai*, há pouca plasticidade fenotípica e a adaptação a ambientes frios se dá por meio do aumento da freqüência do alelo que condiciona o fenótipo escuro (Gibert *et al.*, 1999).

Em espécies do subgrupo *dunni*, Hollocher *et al.* (2000) propuseram que, além das influências ambientais, a seleção sexual ou o reconhecimento sejam fatores importantes na evolução da pigmentação abdominal. Em *D. melanogaster*, a pigmentação das fêmeas pode ser muito importante na determinação de sua atratividade aos machos. Nesta espécie ocorre dimorfismo sexual da pigmentação do abdome, de modo que os machos possuem os últimos tergitos intensamente pigmentados, o que não ocorre com as fêmeas. Foi verificado que fêmeas com coloração de macho são preteridas pelos machos, o que pode ser fundamental para a determinação do dimorfismo sexual (Kopp *et al.*, 2000).

Inversões cromossômicas em Drosophila

Mais da metade das espécies de *Drosophila* já estudadas apresentam polimorfismo de inversões cromossômicas em um ou mais braços cromossômicos (Powell, 1997).

Inversões cromossômicas suprimem a recombinação na região invertida. Isso ocorre porque, quando há *crossing-over* dentro da região invertida em heterozigotos para inversões, os gametas produzidos são anormais, isto é, possuem dois centrômeros ou nenhum centrômero, e possuem duplicações e/ou deleções de genes. Esses gametas são, portanto, inviáveis. Em *Drosophila*, não há redução na fertilidade devida à produção destes gametas inviáveis porque, em geral, o *crossing-over* naturalmente não ocorre em machos. Além disso, nas fêmeas, as células portadoras de cromossomos recombinantes anormais originam os corpúsculos polares. Uma revisão sobre inversões cromossômicas em *Drosophila* está em Powell (1997).

Wright e Dobzhansky (1946) e Dobzhansky (1947) foram os primeiros a estabelecer que diferentes inversões cromossômicas tinham valores adaptativos diferentes e estavam, portanto, sob seleção natural. Uma vez que não ocorre recombinação entre genes contidos em diferentes inversões, é possível que ocorra o acúmulo de alelos coadaptados dentro de uma inversão. Polimorfismos de inversões em *Drosophila* já foram relacionados com várias características determinantes do valor adaptativo, como viabilidade em diferentes estágios de vida (Ruiz *et al.*, 1986), resistência a altas temperaturas (Quintana e Prevosti, 1991) e longevidade e tamanho do corpo (Norry *et al.*, 1995; Rodríguez *et al.*, 1999). Inversões cromossômicas também foram associadas a características comportamentais como, por exemplo, a intensidade da atividade de oviposição (Dahlgaard *et al.*, 2001) e da atividade de vôo durante o dia (Gosteli, 1991). Além disso, também foram relacionadas a características morfológicas: cerdas extras (Garcia-Vázquez *et al.*, 1989; Izquierdo *et al.*, 1991; Das *et al.*, 1994), medidas de tamanho do corpo (Hasson *et al.*, 1992; Norry *et al.*, 1995; Bertrán *et al.*, 1998) e correlações entre medidas de tamanho (Norry *et al.*, 1997). Em *D. mediopunctata*, inversões cromossômicas têm efeito sobre o tamanho e a forma da asa (Bitner-Mathé *et al.*, 1995).

Inversões cromossômicas e supergenes

Ford (1964) considerava que os genes que determinam um polimorfismo conspícuo devem ser mantidos juntos de forma que seus alelos apropriados possam segregar um do outro em um bloco, originando conjuntos alternativos de alelos. Uma das formas de se conseguir isso, o que é chamado de supergene por Darlington e Mather (1949, *apud* Ford, 1964), é fazer com que estes genes estejam proximamente ligados, de modo que raramente ocorra recombinação entre eles. Outra alternativa é incluir os alelos coadaptados em uma inversão cromossômica. Ford (1964) considerava que esta seria a maneira mais eficiente de assegurar que estes alelos segregassem conjuntamente, produzindo apenas as variantes fenotípicas de maior valor adaptativo.

Evidências que indicam a existência de supergenes já foram encontradas em caramujos terrestres *Partula taeniata* (Murray e Clarke, 1976a), *Partula suturalis* (Murray e Clarke, 1976b) e em *Cepaea nemoralis* (Jones *et al.*, 1977). Em borboletas, a existência de supergenes tem sido relacionada principalmente ao mimetismo (Clarke e Sheppard,

1960; Mallet, 1989; Mallet *et. al.*, 1990; Gordon e Das, 1998). Espera-se que supergenes estejam presentes no controle de polimorfismos envolvidos em casos de mimetismo batesiano, em que o modelo é impalatável e o mímico é palatável, porque só é vantajoso ao mímico tornar-se mais conspícuo, caso a semelhança com o modelo impalatável seja suficiente para enganar o predador (Charlesworth, 1994; Mallet e Joron, 1999). Mais recentemente, tem sido mais amplamente aceita a hipótese de que o mimetismo batesiano requer inicialmente uma mutação em um gene principal, que possa gerar já no primeiro passo uma grande semelhança com o modelo, que é posteriormente "aperfeiçoada" por modificadores (Mallet e Joron, 1999). A evolução de um supergene ocorreria então pelo favorecimento de genes modificadores específicos que estivessem proximamente ligados ao gene principal (Charlesworth, 1994). Entretanto, casos de supergenes determinando mimetismo mülleriano (em que ambos o modelo e o mímico são impalatáveis) também têm sido encontrados (Mallet *et al.*, 1990). Em borboletas do gênero *Heliconius,* uma das explicações para este fenômeno é a de que é possível que existam poucas regiões no genoma capazes de afetar o padrão de coloração (Mallet, 1989).

Nos casos citados aqui como exemplos de supergenes, o mecanismo pelo qual se mantêm ligados os alelos apropriados é uma baixíssima distância de recombinação entre eles. Nestes casos, só é possível distinguir um supergene de um único loco principal se forem encontrados recombinantes (Mallet, 1989; Mallet *et al.*, 1990; Gordon e Das, 1998). Não foi possível constatar até agora, a existência de um polimorfismo condicionado por um supergene cuja determinação fosse feita por uma inversão cromossômica, apesar do forte potencial para que isso ocorresse, como já havia sido previsto por Ford (1964).

Drosophila mediopunctata

A espécie estudada neste trabalho é *Drosophila mediopunctata,* pertencente ao grupo *tripunctata*. Este grupo possui 56 espécies, de acordo com Vilela (1992) (desde então, entretanto, outras espécies foram adicionadas), e é o segundo maior grupo neotropical do gênero *Drosophila* em número de espécies, sendo abundante em áreas florestais, principalmente no Sul do Brasil e durante o inverno (Sene *et al.*, 1980; Klaczko, 1995; Saavedra *et al.*, 1995). O nome do grupo se deve à presença de três manchas escuras nos últimos tergitos abdominais. No entanto, nem todas as espécies do grupo apresentam

esse caráter. Em algumas espécies, no último tergito há apenas uma faixa escura e em outras há variação intraespecífica (Frota-Pessoa, 1954).

D. mediopunctata é uma espécie neotropical, que ocorre no Brasil e em El Salvador (Val et al. 1981) Na descrição original da espécie, já havia sido notado por Dobzhansky e Pavan (1943) que as manchas abdominais podem estar presentes ou ausentes. Frota-Pessoa (1954) foi o primeiro a caracterizar a variação do número de manchas escuras no abdome de *D. mediopunctata*, verificando que estes animais podem apresentar: nenhuma mancha, apenas uma mancha no sexto tergito, duas manchas (no quinto e sexto tergito) ou três manchas (no quarto, quinto e sexto tergito). Frota- Pessoa (1954) observou também que as manchas variam quanto ao tamanho e que machos tendem a apresentar manchas maiores e em maior número do que as fêmeas.

D. mediopunctata tem seis pares de cromossomos: cinco acrocêntricos e mais um par que não se politeniza. Os cromossomo II, IV e X apresentam polimorfismo de inversões (Peixoto e Klaczko, 1991; Ananina *et al.* 2002). O cromossomo II é o mais polimórfico e suas inversões podem ser divididas em dois grupos, de acordo com a região do cromossomo: existem oito inversões na região distal (*DA, DI, DV, DS* etc.) e nove na região proximal (*PA0, PB0, PC0, PC1, PC2* etc.) (Ananina *et al.*, 2002). Há um forte desequilíbrio de ligação entre as inversões proximais e distais do cromossomo II, de modo que *PA0* está predominantemente ligada a *DA* enquanto *PC0* está preferencialmente ligada a *DV, DP* e *DS* (Peixoto e Klaczko, 1991).

As freqüências das inversões do cromossomo II se alteram durante o ano, de forma que *PA0* tende a ser mais freqüente nos meses mais frios, enquanto o contrário ocorre para *PC0* (Klaczko, 1995). Existe também uma correlação positiva entre a freqüência de *PA0* e altitude, enquanto a mesma correlação é negativa para *PC0* (Klaczko, 1995). Esses dados parecem indicar que *PA0* está mais adaptada a temperaturas mais baixas e *PC0*, a temperaturas mais altas.

Objetivos

Os objetivos deste trabalho foram

1. Caracterizar a variação no número de manchas abdominais de populações naturais de *Drosophila mediopunctata* de duas localidades (Morro Santana, em Porto Alegre, RS, e Mata Santa Genebra, em Campinas, SP), mais especificamente:
   a) verificar se a variação do padrão de coloração está de acordo com o que já foi estabelecido em outras espécies de *Drosophila* pela variação local de temperatura, ou seja, se quando as temperaturas locais são mais baixas, os fenótipos são mais escuros;
   b) verificar se a variação genética ocorre no mesmo sentido que a variação ambiental, ou seja, se quando as temperaturas locais são mais baixas, as moscas são também geneticamente mais escuras.
2. Verificar se existe uma associação não-aleatória entre inversões do cromossomo II e o polimorfismo de inversões.
3. Verificar se há efeitos de densidade de larvas e temperatura de desenvolvimento sobre o fenótipo de manchas abdominais.

Organização da dissertação

Essa dissertação está organizada da seguinte forma: a esta introdução geral seguem-se dois artigos redigidos na forma em que serão submetidos à publicação (com exceção da posição de tabelas e figuras, que foram distribuídas no texto de forma a facilitar a leitura) e uma conclusão geral.

Este trabalho é parte de uma linha de pesquisa que vem sendo desenvolvida pelo prof. Louis Bernard Klaczko desde a década de 80, na qual são estudados diversos aspectos de *Drosophila mediopunctata.* Este trabalho também é, em parte, fruto da colaboração entre dois grupos de pesquisa: o laboratório do Prof. Louis Bernard Klaczko, meu orientador, e o laboratório da Profa. Vera Valente Gaiesky, da Universidade Federal do Rio Grande do Sul. Desta colaboração resultou o primeiro trabalho que compõe a dissertação, do qual a profa. Vera e Luciano Basso, também da UFRGS, são co-autores. Fazem parte do trabalho que eu

desenvolvi durante o mestrado a análise das populações de *D. mediopunctata* de Campinas e de Porto Alegre, a análise dos padrões de coloração das estirpes com diferentes cromossomos II sobre o mesmo *background* genético e das proles dos cruzamentos entre elas. Dessa forma, estão apresentadas nos artigos, mas não fazem parte de meus trabalhos de mestrado: a análise genética cromossômica do polimorfismo e a produção das estirpes de mesmo fundo genético para o cromossomo II, que foram desenvolvidos pelos demais co-autores dos artigos.

Durante meus estudos, analisei 284 indivíduos coletados, 2065 da prole destes (criados em condições padronizadas), 1900 de estirpes com diferentes cromossomos II sobre o mesmo *background* genético e 13090 das proles de cruzamentos entre estas estirpes (criados em condições padronizadas).

TEMPORAL VARIATION IN THE COLOUR POLYMORPHISM OF TWO NATURAL POPULATIONS OF *DROSOPHILA MEDIOPUNCTATA*.

Luciane M. Hatadani[1], Luciano Basso da Silva[2], Vera L. S. Valente[2]

and Louis B. Klaczko[1†]

[1]Departamento de Genética e Evolução, Instituto de Biologia, Universidade Estadual de Campinas, UNICAMP, [2]Departamento de Genética, Instituto de Biociências, Universidade Federal do Rio Grande do Sul, Porto Alegre, Brazil

ABSTRACT

The *tripunctata* species group of *Drosophila* is the second largest Neotropical group in number of species, being abundant in forests. Most of the species of this group present three dark spots on the fourth, fifth and sixth abdominal tergites, thus the name *tripunctata*. However, in some species, this pattern is variable; such is the case of *Drosophila mediopunctata*. There are flies ranging from zero to three spots. In this study, the abdominal pigmentation patterns of two natural *D. mediopunctata* populations were investigated. Two collections were performed in each of the areas studied. For each collection, the second generation descendants from the collected females were raised in standardised temperature and density conditions, making it possible to determine the influence of these variables on the colour polymorphism. The density, in general, has little effect on the polymorphism. The temperature has an intense effect: flies raised in lower temperatures tend to have more spots, *i.e.* are darker, an effect that also occurs in the other *Drosophila* species. The colour pattern of the flies collected from the field presented the expected variation according to the variation of the local temperatures in both sites, darker flies were found in collections at lower temperatures. Contrary to what happens in other *Drosophila* species, the genetic variation occurred in the opposite direction to that determined by the environmental variation, a counter-gradient pattern, suggesting the action of natural selection.

INTRODUCTION

Ever since Cain and Sheppard (1950) showed that the pigmentation patterns on the *Cepaea nemoralis* shells are under selection, it has been supposed that most conspicuous polymorphisms are under natural selection. Sewall Wright (1978) believed these polymorphisms were not typical of the variation which evolution depends upon. Nonetheless, he agreed that the conspicuous polymorphisms are particularly favourable to the study of the action of natural selection and can represent a unique opportunity for the study of adaptation.

In the *Drosophila* genus, despite the almost 2000 species (Powell, 1997), few of the conspicuous variation cases have been studied, among these are the study of the abdominal coloration in *D. polymorpha* (Da Cunha, 1949; Heed and Blake, 1963; Martinez and Cordeiro, 1970) and *D. kikkawai* (Freire-Maia, 1964, Gibert *et al.*, 1999), the coloration of the thorax in *D. lebanonensis* (Pipkin, 1962) and the thorax trident coloration in *D. melanogaster* and *D. simulans* (Capy *et al.*, 1988). In these last two species, clines were found in which, the higher the latitude, the greater the pigmentation. This similarity among different species constitutes a strong argument in favour of the adaptive nature of the cline.

In general, the influence of the temperature on the pigmentation in *Drosophila*, in regard to the phenotypic plasticity, leads to lighter coloured flies in high temperatures and darker flies in lower temperatures. The most common adaptive explanation is that darker phenotypes have an advantage in colder regions as they absorb more heat, consequently increasing the internal temperature (Gibert *et al.*, 1996, Capy *et al.*, 1988). Evidence to support this hypothesis was found for *D. melanogaster* and *D. simulans* (Gibert *et al.*, 1996), in which genetically darker flies were found in colder places, suggesting that darker phenotypes are well adapted to cold environments. The same hypothesis, which involves thermorregulation, has been proposed to explain the coloration patterns of several other animal species, such as butterflies (Watt, 1968), tortoises (Willemsen and Hailey, 1999), snakes (Gibson and Falls, 1979) and frogs (Lillywhite *et al.*, 1998).

The *tripunctata* group is the second largest Neotropical *Drosophila* species group in number of species, being abundant in forests, especially in southern Brazil and during the winter (Sene *et al.*, 1980; Klaczko, 1995; Saavedra *et al.*, 1995). Most of the species in this group present dark spots on the last abdominal tergites (Frota-Pessoa, 1954). Dobzhansky

and Pavan (1943) had already noticed that in *Drosophila mediopunctata*, these spots could be present or not. Frota-Pessoa (1954) was the first to characterise the variation of the dark spots on the abdomen of *D. mediopunctata*, showing that these animals could present: no spots, only one spot (on the sixth tergite), two spots (on the fifth and sixth tergites) or three spots (on the fourth, fifth and sixth tergites). In a sample of 50 males and 50 females from Cantareira (near the city of São Paulo, Brazil), collected in March 1953, this author found an average of 0.74 ± 0.12 spots on the females and 1.36 ± 0.13 spots on the males. Frota-Pessoa also observed that the size of the spots also varies, and that, in general, males tend to present more and bigger spots than the females.

In this study, we describe the variation in the number of abdominal spots of natural populations of *Drosophila mediopunctata* from two localities (Morro Santana in Porto Alegre, RS, and Mata Santa Genebra in Campinas, SP, both in Brazil). The objectives of this study are: (1) verify if the coloration pattern variation is in agreement with what has already been established in other species according to the local temperature variation, in other words, if when the local temperatures are lower, the phenotypes are darker; (2) verify the existence of genetic variation and if it occurs in the same direction as that determined by the environmental variation, that is, if when the local temperatures are lower, the flies are also genetically darker, (3) verify effects of sex, temperature and density on the color polymorphism.

MATERIALS AND METHODS

Collections

Two collections were made in Morro Santana (30°02'S-51°14''W), in Porto Alegre in the state of Rio Grande do Sul (Brazil) an area of secondary forest in the humid subtropical climate zone (Basso da Silva and Valente, 2000). The first collection was made in May of 1999 and the second in October of 2000.

Two other collections were made in the Mata Santa Genebra in August and September of 2000. Mata Santa Genebra (22°44' 45''S, 47°06'33''W) is a semi-deciduous forest located in the city of Campinas, São Paulo, Brazil and is located in an area of seasonal tropical climate (Morellato and Leitão Filho, 1995).

All of the collected flies were classified alive with regard to their colour polymorphism, according to the number of spots on the tergites, from 0 to 3 spots (Figure 1). In flies with 3 spots, the spot on the fourth tergite sometimes is blended into the posterior pigmented band. In those cases, the phenotype was scored as "**3D**", but the analysis is done by considering this pattern as having three spots.

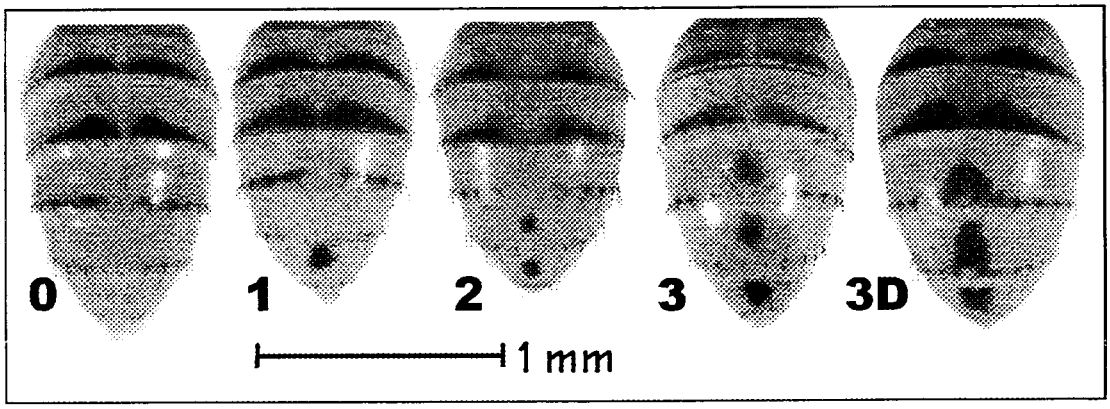

Figure 1: Phenotypes of dark spots on the tergites of *D. mediopunctata*, the number of spots is marked at the left of the photograph of each abdomen; **3D** represents the three spots pattern with the spot on the fourth tergite blended into the intersegmentary band.

Obtaining and analysing flies under standardised conditions

Each collected female was placed in a tube with culture medium to lay individually. Of the offspring of each female which produced sufficiently, equal numbers of males and virgin females were separated (about six couples per group of offspring), which after ten days were placed in the same bottle, where first stage larvae were collected from. These larvae were distributed in tubes with 10ml of culture medium (8% yeast, 2% sugar, 2.5% agar, 0.003% nipagin). Four different conditions were established for the development of the larvae: 100 larvae per tube, at 20°C; 10 larvae per tube, at 20°C; 100 larvae per tube, at 16.5°C; 10 larvae per tube, at 16.5°C.

The flies that emerged from these tubes were classified alive according to their number of spots, four days after the day they emerged, period after which the coloration had stabilised.

Statistical analysis, genetic and environmental variation

The results of the collections and of flies raised under standardized conditions were analysed by CATMOD (SAS Institute, 1985). Since all flies raised under the same standardized condition were assumed to develop under equal environmental conditions, the differences observed were considered to be originated by genetic variation. On the other hand, differences found among collected flies, which were subject to unknown environmental conditions, were considered to be originated both by genetic and environmental variation.

RESULTS

Collected Flies

Of the collections done in Porto Alegre, it can be observed that the individuals collected in October of 1999 were of the darker phenotype, that is, with more spots on the tergites than those collected in May of 2000 (Figure 2). The average was 2.77±0.10 spots per male and 2.57±0.23 spots per female for the collection of October 1999, while for the May 2000 collection, the averages were 2.16±0.09 and 1.08±0.36 for males and females respectively (Figure 3), constituting a significant difference between the two collections (Table 1).

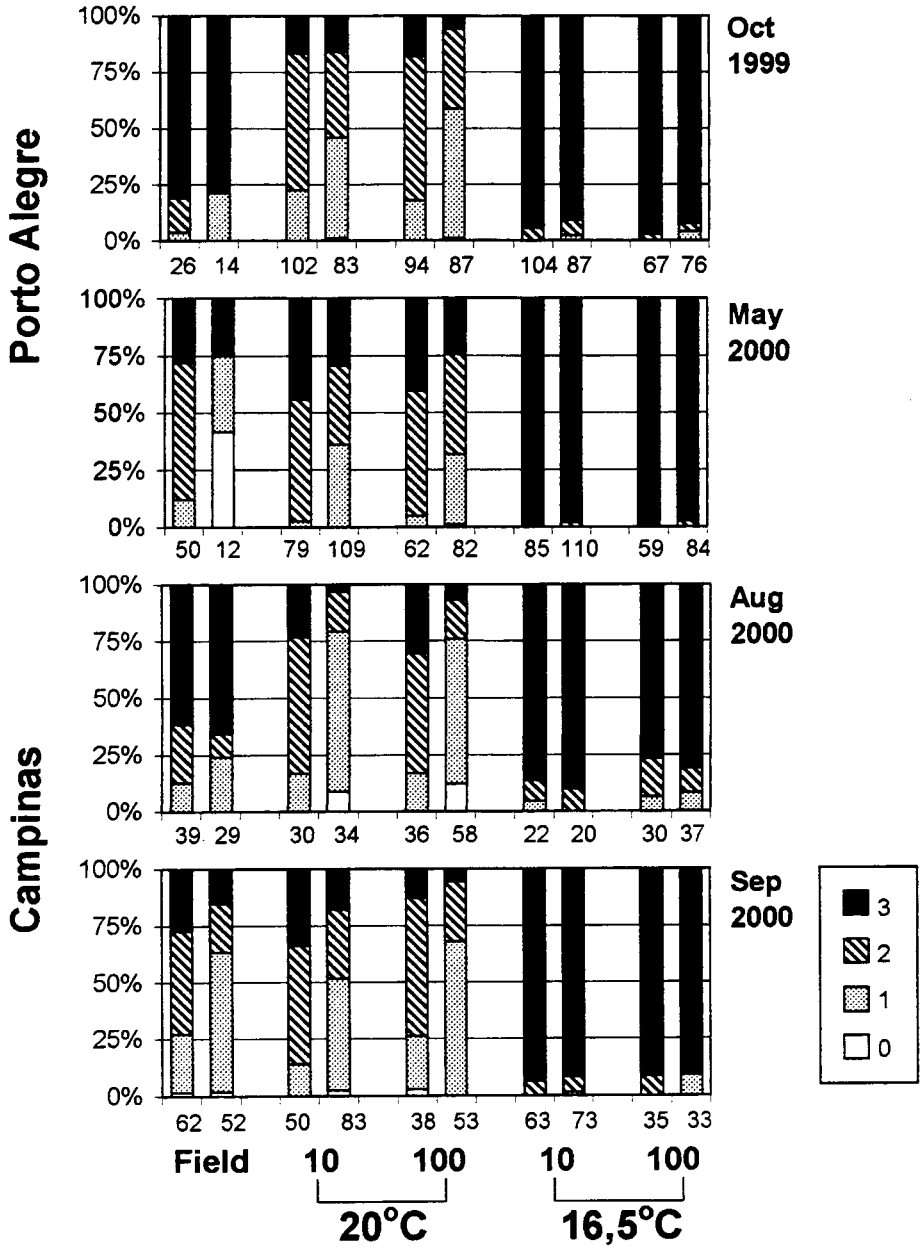

Figure 2: Proportions among the classes of abdominal coloration observed in each of the collections for the animals from the field and those raised under controlled temperature and density environments. The first pair of percentage bars indicates the proportions of the phenotypes among the collected flies, the second and third pairs represent flies raised under density of 10 larvae per tube and 100 larvae per tube, respectively, both kept on 20°C; the fourth and fifth pairs represent flies raised under density of 10 larvae per tube and 100 larvae per tube, respectively, both kept on 16.5°C. In every pair of bars, the first indicates the proportions among the males, and the second, among the females. The number bellow each bar, indicates the number of individuals sampled in each case.

A significant difference was also observed between the two collections done in Campinas (Table 1). In this case, the flies collected in August of 2000 presented darker phenotypes (average of 2.49±0.12 spots per male and 2.41±0.16 spots per female) than those collected in September of the same year for which the average was 1.98±0.10 per male and 1.50±0.11 per female (Figure 3). The sex has no significant effect in any of the studied localities.

Table 1: CATMOD (SAS Institute, 1985) testing the location effect, collection date and sex among the collected flies. Significant values of P are in bold.

Locality	Source	d.f.	Chi-square	Probability
Porto Alegre	Intercept	1	0.39	0.5306
	Collection date	1	18.78	**<0.0001**
	Sex	1	0.07	0.7926
	Likelihood ratio	1	0.00	0.9867
Campinas	Intercept	1	5.03	**0.0249**
	Collection date	1	28.53	**<0.0001**
	Sex	1	0.86	0.3537
	Likelihood ratio	1	1.69	0.1930

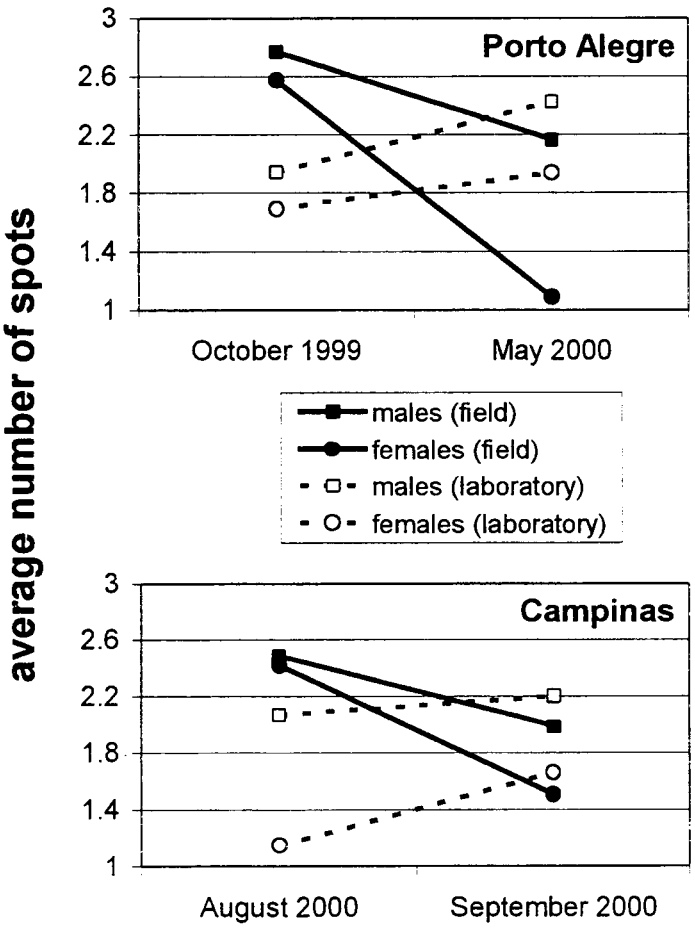

Figure 3: Variation of the average number of spots on the tergites between the two collection locations, for the collected individuals and those raised in the laboratory, in a 10 larvae per tube density at 20°C.

Flies raised in standardised conditions

When considering the animals raised under a controlled temperature and density, from both locations, there is a noticeable effect of the temperature, in the sense that those that were raised at 16.5°C have more spots (Figure 2). The effect of temperature is so intense that almost all flies raised in this temperature have three spots. Thus, it is more informative to concentrate the rest of the analysis on flies raised at 20°C.

It is possible to detect a significant difference between the two collections of Porto Alegre, in such a way that the individuals raised in the laboratory derived from the May 2000 collection are from phenotypes with more spots (Table 2). Particularly for the animals raised at 20°C, 10 larvae per tube, the average number of spots on the males was 1.94±0.06 in the October collection and raised to 2.43±0.06 in the May collection (Figure 3). Similarly, the average for the females rose from 1.69±0.08 to 1.94±0.08. No interactions were observed in this location.

In Campinas, the difference between the collections was not significant (Table 2). There is however, a significant interaction between collection date and density. If the data from Campinas is separated by density, the difference between collections is significant among flies raised on vials containing 10 larvae but is not significant among flies raised on vials containing 100 larvae. The averages of the animals raised at 20°C, 10 larvae per tube, were, for the August collection, of 2.07±0.12 for the males and 1.14±0.10 for the females, while for the September collection, these averages increased to 2.20±0.10 and 1.66±0.09, for males and females, respectively (Figure 3).

Table 2: CATMOD (SAS Institute, 1985) testing the location effect, collection date, density and sex among the animals raised at 20°C in the laboratory from each location. Significant values of P are in bold.

Locality	Source	d.f.	Chi-square	Probability
Porto Alegre	Intercept	1	160.64	**<0.0001**
	Collection date (C)	1	36.44	**<0.0001**
	Sex (S)	1	10.64	**0.0011**
	Density (D)	1	2.49	0.1142
	C x S	1	0.18	0.6736
	C x D	1	0.30	0.5840
	S x D	1	1.66	0.1972
	Likelihood ratio	1	1.99	0.1580
Campinas	Intercept	1	108.47	**<0.0001**
	Collection date	1	0.59	0.4440
	Sex	1	18.13	**<0.0001**
	Density (D)	1	1.12	0.2902
	C x S	1	233	0.1271
	C x D	1	6.84	**0.0089**
	S x D	1	0.03	0.8622
	Likelihood ratio	1	0.16	0.6903

The difference between sexes is significant in both locations (Table 2), but the density effect was not significant.

Had there not been a significant difference between the collections for the animals raised under controlled conditions, it would be possible to conclude that the difference observed among the field individuals between the collection dates was purely environmental. The difference found, however, is not only significant, but also contrary to the difference observed among the field flies, in Porto Alegre as well as in Campinas.

Field Temperatures

Since the temperature has such a strong effect on the phenotype of the abdominal spots for *D. mediopunctata*, it becomes interesting to analyse the temperature variation in the collection locations over the period which the flies were collected. Moreover, the genetic differences found are contrary to the phenotypic differences observed among the collections performed in both locations. Thus, the presence of an environmental factor causing the phenotypic variations among collections is necessary.

According to temperature data made available by the *8° Distrito de Meteorologia, Instituto Nacional de Meteorologia, Ministério da Agricultura e Reforma Agrária* (Figure 4), one can observe that the temperatures measured immediately prior to the first collection in Porto Alegre (performed in October of 1999) are in fact lower than the temperatures registered prior to the second collection (May of 2000). Specifically, the average temperatures for the 30 days previous to each collection were 17.7±0.6 °C and 20.8±0.5°C, for the first and second collection respectively. So, the hypothesis that the temperature was an important factor in the determination of the differences observed among the individuals collected on each of the two dates seems plausible, since the temperature variation occurred in the manner predicted by the phenotypic pattern observed.

For Campinas, the period immediately prior to the August collection date registered lower temperatures than those registered prior to the September collection (figures provided by *CEPAGRI, Centro de Ensino e Pesquisas em Agricultura, UNICAMP*) (Figure 4). The average temperatures for the thirty days prior to each collection were 15.6±0.5°C for the August collection and 22.0±0.4°C for September. Therefore, in the

same manner as occurred in Porto Alegre, the phenotypic variation observed among the individuals collected between August and September occurred as expected, according to the temperature variation in the same period.

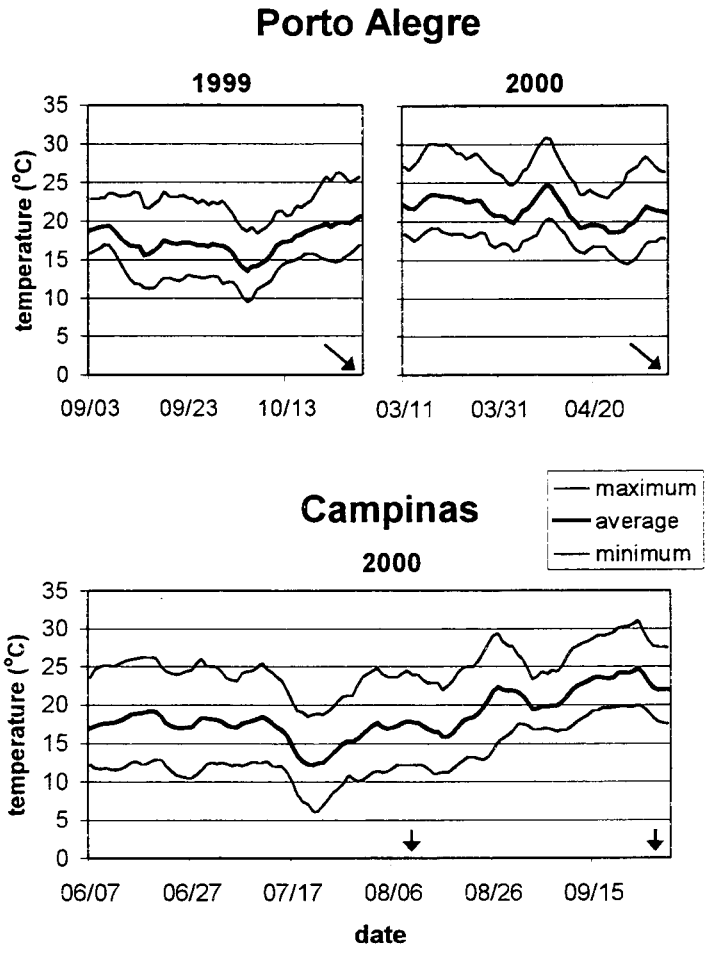

Figure 4: Temperature variation in Porto Alegre and Campinas in the periods preceding each of the collections (7-day sliding averages). The date of each collection is pointed out with an arrow.

DISCUSSION

The number of spots of flies raised in the laboratory under 20°C from both locations present a significant difference between the sexes. Wild-caught flies did not differ significantly between sexes, but this is probably due to the grouping used in the statistical test, which gathered in the same class flies with 0, 1 and 2 spots. This would

cause the difference between sexes to disappear because, within classes 1 and 2, males tend to have two spots while females have one spot (Figure 2). Thus, our data are similar to the initial observation by Frota-Pessoa (1954) that the male *D. mediopunctata* present, in general, more spots on the abdominal tergites than the females.

Among the flies raised in the laboratory, the density was not important. Freire-Maia (1964) suggested that the frequencies of the abdominal coloration polymorphism phenotypes in *D. kikkawai* depend upon the conditions of the culture medium, being that the larvae density during their development is one of the variables involved. This does not seem to be the case of *D. mediopunctata.*

The effect of the temperature was of greater importance among the laboratory-raised animals, increasing the number of spots on the flies raised in lower temperatures. The increase in pigmentation with the lowering of the temperature was also observed in *D. kikkawai* (Freire-Maia, 1964, Gibert et al., 1999), *D. melanogaster* and *D. simulans* (Capy et al., 1988, Gibert et al., 1996) and has been interpreted as an adaptation in the sense that the darker phenotypes are more advantageous in lower temperatures (thermal budget hypothesis). This hypothesis is based on the fact that the greater the pigmentation, the greater the absorption of the sun's radiation, thus raising the animal's temperature and consequently, its metabolism. On the other hand, lighter phenotypes are more advantageous in higher temperatures as they lower the chances of overheating (Watt, 1968). Therefore, according to this hypothesis, one would expect to find darker flies in colder environments and lighter flies in hotter environments.

Indeed, in both locations studied, the collections that had been preceded by colder periods (October of 1999 in Porto Alegre and August of 2000 in Campinas) presented flies with significantly darker phenotypes than those collected after warmer periods (May of 2000 in Porto Alegre and September of 2000 in Campinas). This is in agreement, with what would be expected given the temperature variation, with the *thermal budget hypothesis*

Another argument that reinforces the hypothesis that the pigmentation in *Drosophila* is an adaptive character related to the thermoregulation has been the existence of latitudinal clines in which genetically darker flies are found in higher latitudes, while genetically lighter flies are found in lower latitudes. This way, the phenotypic variation

caused by the environmental variation is reinforced by the genotypic variation due to natural selection. This phenomenon was observed in the abdominal pigmentation of *D. melanogaster* (Das, 1995) and for the trident coloration of both *D. melanogaster* and *D. simulans* (Capy et al., 1988). The parallelism between the clines for the same character in the last two species reinforces the argument in favour of its adaptive character. In *D. kikkawai*, darker phenotypes were also observed in locations with a colder winter, which occurs by means of a change in the allelic frequency in the locus which controls the polymorphism (Gilbert et al., 1999).

The most interesting and surprising result of this study was that in both locations, the genetic variations occurred in the opposite direction to the environmental influences. The similarity between the two locations strongly suggests the effect of natural selection. Falconer (1990) uses the expression *antagonistic selection* for a situation in which natural selection and the environment affect the character in opposite directions. According to Conover and Schultz (1995), this kind of natural selection would make the distribution of the genotypes among natural populations in such a way that the genetic and environmental influences on the phenotype would oppose each other by means of a gradient. This would reduce the phenotypic variation and create a genotype distribution pattern called *counter-gradient variation.* Counter-gradient variation had already been observed for body size in some species of *Drosophila* (Levins, 1969), even though it has not yet been reported for pigmentation.

This variation pattern could occur due to the presence of trade-offs with components of the adaptive value, or yet, if natural selection acts in opposite directions upon a single character on opposite ends of the environmental gradient (Conover and Schultz, 1995).

There is a possibility that, even if a greater pigmentation is more advantageous in lower temperatures, the pigment production occurs at the expenses of other components of the adaptive value. Windig (1999) suggested that, in a species of butterflies, there could be a trade-off between the production of melanin and life history traits.

Another possibility to be considered is that the action of natural selection occurs in opposite directions on the extremities of the environmental gradient. Sexual selection may be one of the selective forces which act upon pigmentation. In species of the *dunni*

subgroup, sexual selection, aside from the environmental influences, seems to be an important factor in the abdominal pigmentation (Hollocher et al., 2000). In *D. melanogaster*, the pigmentation of females can be very important in determining their attractiveness toward males, which can be of great importance to the determination of sexual dimorphism.

Another possible situation involving sexual selection would be if the male or female preferences were altered by the environmental conditions. These kinds of seasonal changes were observed in coccinellid beetles of the genus *Harmonia*, causing cyclic variations in the frequencies of the colour polymorphism in natural populations (Osawa and Nishida, 1992).

On the other hand, perhaps the simplest explanation is that natural selection is constantly favouring a single phenotype. In this case, when the environment causes a variation in one direction, distancing the phenotype from its adaptive optimum, natural selection would act in the opposite direction, which would be enough to cause the observed pattern. The possibility that several of these factors can be acting together, rather than just one of them, cannot be discarded. None of these hypotheses, however, can be tested with the data available. For this, it is necessary to perform experiments and to obtain data with the specific objective of testing each one of them. For this reason, we are continuing our research to understand the temporal variation of the abdominal colour patterns in *Drosophila mediopunctata*, especially with respect to factors that cause genetic variation.

ACKNOWLEDGEMENTS

It is a pleasure to thank Carlos A. C. Andrade, Galina Ananina, Hermes F. Medeiros and Horácio Montenegro for the decisive help in field collections; Wilma N. Souza, Laércio Santana, Andre V. Freitas for technical help; and Edward Hogan for correcting the English version. Profs. Jean David and Brigitte Moreteau gave insightful suggestions. Supported by grants from: Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq); Coordenação de Aperfeiçoamento de Pessoal de Ensino Superior (CAPES); Fundação de Amparo à Pesquisa de São Paulo (FAPESP); Fundação de Amparo à Pesquisa do Rio Grande do Sul; Fundo de Apoio ao Ensino e Pesquisa (FAEP-UNICAMP) and Propesq-UFRGS.

REFERENCES

BASSO DA SILVA, L.; VALENTE, V. L. S. A temporal analysis of sexual activity in a natural population of *Drosophila willistoni*. **Hereditas**, n. 133, p. 211-216. 2000.

CAIN, A.J.; SHEPPARD, P. M. Selection in the polymorphic land snail *Cepaea nemoralis*. **Heredity**, n. 4, *p. 275-94*. 1950.

CAPY, P.; DAVID; J. R.; ROBERTSON, A. Thoracic trident pigmentation in natural populations of *Drosophila simulans*: a comparison with *D. melanogaster*. **Heredity**, n. 61, p. 263-268. 1988.

CONOVER, D. O.; SCHULTZ, E. T. Phenotypic similarity and the evolutionary significance of countergradient variation. **TREE**, n. 10, p. 248-252. 1995.

DA CUNHA, A. B. Genetic analysis of the polymorphism of color pattern in *Drosophila polymorpha*. **Evolution**, n. 3, p. 239-251. 1949.

DAS, A. Abdominal pigmentation in *Drosophila melanogaster* females from natural Indian populations. **J. Zool. Sys. Evol. Res.** , n. 33, p. 84-87. 1995.

DOBZHANSKY, T.; PAVAN, C.. Studies on Brazilian species of *Drosophila*. **Bol. Facul. Fil. Ciên e Letr. Univ. S. Paulo**, n. 36. 1943.

FALCONER, D.S. Selection in different environments: effects on environmental sensitivity (reaction norm) and on mean performance. **Genet. Res.** , n. 56, p. 57-70. 1990.

FREIRE-MAIA, N. Segregational load in *Drosophila kikkawai* I. Crossing experiments. **Genetics**, n. 50, p. 211-219. 1964.

FROTA-PESSOA, O. Revision of the *tripunctata* group of *Drosophila* with description of fifteen new species (Drosophilidae, Diptera). **Arq. Mus. Para.** , n. 10, p. 253-304. 1954.

GIBERT, P. et al. Growth temperature and adult convergence of reaction norms in sympatric populations? **Evolution**, n. 50, p. 2346-2353. 1996.

GIBERT P. et al. Phenotypic plasticity of abdominal pigmentation in *Drosophila kikkawai*: multiple interactions between a major gene, sex, abdomen segment and growth temperature. **Genetica**, n. 105, p.165-176. 1999.

HEED, W. B.; BLAKE, P. R.. A new color allele at the *e* locus of *Drosophila polymorpha* from northern South America. **Genetics**, n. 48, p. 217-234. 1963.

HOLLOCHER, H.; HATCHER, J. L.; DYRESON, E. G. Evolution of abdominal pigmentation differences across species in the Drosophila dunni subgroup. **Evolution**, n. 54, p. 2046-2056. 2000.

KLACZKO, L.B. 1995. In: Levine,L. (Ed.) **Genetics of Natural Populations: the Continuing importance of Theodosius Dobzhansky**. New York: Columbia University Press. Pp. 140-153.

KOPP, A.; DUNCAN, I.; CARROLL, S. B. Genetic control and evolution of sexually dimorphic characters in Drosophila. **Nature**, n. 408, p. 553-562. 2000.

LEVINS, R. Thermal acclimation and heat resistance in *Drosophila.* **Am. Nat.**, n. 103, p. 483-499. 1969.

LILLYWHITE, H. B. et al. Basking behavior, sweating and thermal ecology of the indian tree frog, *Polypedates maculatus*. **J. Herpetol.,** n. 32, p. 169-175. 1998.

MARTINEZ, M.N.; CORDEIRO, A. R. Modifiers of color pattern genes in *Drosophila polymorpha*. **Genetics**, n. 64, p. 573-587. 1970.

MORELATTO, P.C.; LEITÃO-FILHO, H. F. (Eds.) **Ecologia e preservação de uma floresta tropical urbana: Reserva de Santa Genebra**. Campinas: Editora da Unicamp, 1995.

OSAWA, N.; NISHIDA, T. Seasonal variation in elytral colour polymorphism in *Harmonia axyridis* (the ladybird beetle): the role of non-random mating. **Heredity**, n. 69, p. 297-307. 1992.

PIPKIN, S.B. Mesonotal color polymorphism in *Drosophila l. lebanonensis*. **Genetics**, n. 47, p. 1275-1290. 1962.

POWELL, J. R. **Progress and Prospects in Evolutionary Biology. The Drosophila Model**. New York: Oxford University Press. 1997.

SAAVEDRA, C. C. R. et al. A descriptive and analytical study of our neotropical drosophilid communities. **J. Zoo. Sys. Evol. Res.**, n. 33, p. 62-74. 1995.

SAS Institute Inc. SAS/STAT 1988. SAS/STAT™ User's Guide, Release 6.03 Edition. Cary, N.C. SAS Institute Inc., 1988. 1028 pp.

SENE, F. M. et al. Preliminary data on the geographical distribution of *Drosophila* species within morphoclimatic domains of Brazil. **Papéis Avuls. Zoo. S. Paulo**, n. 33, p. 315-326. 1980.

VAL, F. C.; VILELA, C. R.; MARQUES, M. D. In: Ashburner M., Carson HC, Thompson, Jr JN. (eds.), **The genetics and Biology of Drosophila**. Vol. 3a. London: Academic Press. 1981.

WATT, W. B. Adaptive significance of pigment polymorphisms is *Colias* butterlies I.Variation of melanin pigment in relation to thermoregulation. **Evolution**, n. 22, p. 437-458. 1968.

WINDIG, J. J. Trade-offs between melanization, development time and adult size in *Inachis io* and *Araschnia levana* (Lepidoptera: Nymphalidae). **Heredity**, n. 82, p. 57-68. 1999.

WILLEMSEN, R. E.; HAILEY, A. A latitudinal cline of dark plastral pigmentation in the tortoise *Testudo hermanni* in Greece. **Herpetol. J.,** n. 9, p. 125-132. 1999.

WRIGHT, S. **Evolution and the Genetics of Populations**. Vol 4. Chicago: The University of Chicago Press. 1978.

COLOR POLYMORPHISM IN *DROSOPHILA MEDIOPUNCTATA*: GENETIC (CHROMOSOMAL) ANALYSIS AND NON-RANDOM ASSOCIATION WITH CHROMOSOME INVERSIONS.

Luciane M. Hatadani[1], João C. R. Baptista[2], Wilma N. de Souza[1] and Louis B. Klaczko[1†]

[1]Departamento de Genética e Evolução, Instituto de Biologia, Universidade Estadual de Campinas (UNICAMP), Campinas, SP, Brazil [2]Departamento de Genética, Instituto de Biologia, Universidade Federal do Rio de Janeiro. (UFRJ), Rio de Janeiro, RJ, Brazil

ABSTRACT

Chromosome inversions can accumulate coadapted alleles since recombinants between genes within different inversions are not formed. Such inversions could act as supergenes and may be associated with conspicuous polymorphisms. In *Drosophila mediopunctata,* there is a color polymorphism in which three dark spots are present on the fourth, fifth, and sixth tergites. In this paper, we present a genetic (chromosomal) analysis showing that the second chromosome is the major contributor to the genetic determination of this color polymorphism. Since the second chromosome shows inversion polymorphism, we also examined the influence of the inversions on this character. Strains in which different second chromosomes were placed on the same genetic background were used. The number of abdominal spots in these strains and in the offspring of crosses between them was determined at specified temperatures and densities. There was a non-random association between the number of spots and inversions *PA0* and *PC0*. In addition, analysis of the crosses showed that the character was affected significantly by sex and temperature. Since previous studies have shown that *PA0* is adapted to lower temperatures, whereas *PC0* is favored by higher temperatures and since temperature has a marked influence on the color polymorphism, these inversions may involve the accumulation of genes adequate for each temperature, including genes that are related to the number of abdominal spots.

INTRODUCTION

The adaptive nature of chromosome inversions in *Drosophila* has been accepted since Dobzhansky's classic work (Wright and Dobzhansky, 1946; Dobzhansky, 1947), which first established that different chromosomal arrangements were under natural selection. Dobzhansky (1970) believed that inversions could be considered supergenes which accumulated coadapted alleles. Ford (1964) thought that including coadapted alleles in a chromosome inversion was the most effective way to ensure that they would segregate as a block to produce only higher fitness phenotypic variants. Ford also proposed that polymorphic adaptations occurred mainly through this genetic architecture in which genes are tightly linked so that they act as a single unit. More recently, inversion polymorphisms in *Drosophila* have been associated with several fitness-related characters (Ruiz *et al.*, 1986; Quintana and Prevosti, 1991; Rodríguez *et al.*, 1999), behavior (Dahlgaard *et al.*, 2001) and morphological characters (Vázquez *et al.*, 1989; Garcia- Izquierdo *et al.*, 1991; Hasson *et al.*, 1992; Das *et al.*, 1994; Bitner-Mathé *et al.*, 1995; Norry *et al.*, 1995, 1997; Bertrán *et al.*, 1998).

There are presently about 2000 known species of *Drosophila* (Powell, 1997). Yet, only few cases of color polymorphism have been examined in this genus. One of the best studied cases is *D. polymorpha*. Da Cunha (1949) identified one locus and two alleles (with no dominance) which determined the abdomen color pattern polymorphism in this species. Heed and Blake (1963) later found another allele, and Martinez and Cordeiro (1970) discovered that modifiers segregated independently from the major locus. In *D. kikkawai,* abdominal color polymorphism is determined by one locus and two alleles (Gibert *et al.*, 1999), whereas in *D. lebanonensis* the color of the thorax is controlled by one locus (two alleles) and modifiers (Pipkin, 1962).

The *tripunctata* group is the second largest neotropical group in number of species, being particularly abundant in forested areas of southern Brazil during the winter (Sene *et al.*, 1980; Saavedra *et al.*, 1995). The group name *tripunctata* is derived from the presence of three dark spots on the last tergites of the abdomen. However, not all species show this trait. In some species, there is only a dark band on the last tergite instead of three spots, while in others there is intraspecific variation (Frota-Pessoa, 1954). Such is the case of *D. mediopunctata,* a species distributed from southern South America to Central

America. The abdominal color pattern in *D. mediopunctata* varies considerably, from no spots to three dark spots, on the fourth, fifth and sixth abdominal tergites. In addition to number, these spots also vary in size. Males tend to have more and bigger spots than females (Frota-Pessoa, 1954).

Drosophila mediopunctata has six pairs of chromosomes: five acrocentrics and a dot that does not undergo polytenization. The X, second and fourth chromosomes are polymorphic for inversions. The second chromosome is the most polymorphic and its inversions can be divided in two groups according to the region: there are eight inversions in the distal region (*DA, DP, DS, DV* etc.) and nine in the proximal region (*PA0, PB0, PC0, PC1* etc.) (Peixoto and Klaczko, 1991; Ananina *et al.*, 2002). There is intense linkage disequilibrium between distal and proximal inversions. For example, *DA* is associated with *PA0*, with a D' value of 0.98; *DP* is associated with *PC0* (D' = 0.97); *DS* with *PC0* (D' = 0.95); and *DV* with *PC0* (D' = 0.63) (Peixoto and Klaczko, 1991).

In this paper, we present a genetic (chromosomal) analysis showing that one of the chromosomes (the second chromosome) has a fundamental role in determining color polymorphism in *D. mediopunctata.* Examination of the influence of inversions of this chromosome on the character revealed a non-random association between inversions *PA0* and *PC0* and the number of spots.

MATERIALS AND METHODS

The color polymorphism

The number of spots varied from none to one (on the sixth tergite), two spots (on the fifth and sixth tergites) and three spots (on the fourth, fifth and sixth tergites) (Frota-Pessoa, 1954). In flies with three spots, the spot on the fourth tergite sometimes merges with the posterior pigmented band. In those cases, the phenotype was scored as "3D", but the entire analysis was done by considering this pattern as having three spots.

The number of spots was determined in live flies, four days after emergence, when the abdominal pigmentation had stabilised.

Genetic analysis

Strains

ITC-29I. Strain selected for zero spots and brother-sister crossed for over 20 generations.

NA. Marker strain (Carvalho and Klaczko, 1993) carrying the visible mutations *Δ* (*Delta*), *Im* (*Impar*), *cr* (*coral*) and *al* (*alfinete*) on chromosomes *II*, *III*, *IV* and *V*, respectively, and sharing its X and Y chromosomes with strain CR27A.

CR27A. Marker strain carrying the dominant visible mutations *Δ-5* (*Delta-5)* on chromosome *II*, and the recessive mutations *cb* (*cabernet*), *cr* (*coral*) and *al* (*alfinete*) on chromosomes *III*, *IV* and *V*, respectively. This strain shared its X and Y chromosomes with strain CR27B.

CR27B. Marker strain carrying the visible mutations *cb* (*cabernet*), *cr* (*coral*) and *al* (*alfinete*) on chromosomes *III*, *IV* and *V*, respectively, and sharing its X and Y chromosomes with strain CR27A.

In strain ITC-29I, the zero spot phenotype was fixed whereas in the remaining strains the three spot phenotype was fixed.

Detection of the effect of the sex chromosomes

Reciprocal crosses were done between strains ITC-29I and NA to detect the presence of genetic factors that could determine the color polymorphism on the sex chromosomes. If the X or the Y chromosome affected the character, then male offspring of one cross should be different from the males of the other. The female offspring served as a control and were expected to have the same distribution of phenotypes since they had the same autosomes and sex chromosomes in both reciprocal crosses. This would not happen only if there was maternal inheritance.

Genetic (chromosomal) analysis

In order to evaluate the effect of each chromosome on the color polymorphism, several crosses were made. First, females from strain ITC-29I (phenotype 0, for dark spots

on the abdomen) were crossed with males from strain CR27A. This strain is fixed to phenotype 3 and has a recessive lethal mutation with a dominant visible effect (*Delta-5, Δ-5*) in heterozygosis on the second chromosome. The strain is homozygous for recessive mutations on the third, fourth and fifth chromosomes. Male offspring with phenotype Δ were backcrossed with strain CR27B. This strain carries the same mutations on the third, fourth and fifth chromosomes as CR27A, but is homozygous for the wild allele of Δ-5. The offspring was raised under controlled temperature (16.5°C) and density (20 larvae/vial). Since there is no crossing-over in males of *D. mediopunctata*, it is possible to assess the influence of each of the marked chromosomes. If the distribution of phenotypes is independent of the genotype of the markers then there is no influence of the chromosome, otherwise the chromosome carries at least one factor that determines the character.

Strains carrying different inversions on the same genetic background.

Marker strains used to produce strains with the same genetic background

CR26A. Marker strain carrying the recessive mutations *mt* (*merlot*), *cr* (*coral*) and *al* (*alfinete*) on chromosomes *II, IV* and *V*, respectively. This strain shares its X and Y chromosomes with strains CR26B, CR26 C and CR26J. The second chromosome karyotype is *DV-PC0/DV-PC0*.

CR26B. Marker strain carrying the dominant visible mutation Δ (*Delta*) on chromosome *II,* and the recessive mutations *mt* (*merlot*), *cr* (*coral*) and *al* (*alfinete*) on chromosomes *II, IV* and *V*, respectively. The second chromosome karyotype is *DV-PC0/DV-PC0*.

CR26C. Marker strain carrying the dominant visible mutation *Im* (*Impar*) on chromosome *III,* and the recessive mutations *mt* (*merlot*), *cr* (*coral*) and *al* (*alfinete*) on chromosomes *II, IV* and *V*, respectively. The second chromosome karyotype is *DV-PC0/DV-PC0*.

CR26J. Marker strain carrying the dominant visible mutation *Δ-5* (*Delta-5*) on chromosome *II,* and the recessive mutations *mt* (*merlot*), *cr* (*coral*) and *al* (*alfinete*) on chromosomes *II, IV* and *V*, respectively. The second chromosome karyotype is *DA-PA0/DV-PC0*.

Strains CR26A, CR26B, CR26C and CR26J share their sex chromosomes.

Formation of strains with different second chromosomes on the same genetic background

To test whether there was a non-random association between second chromosome inversions and the phenotypes of dark abdominal spots we produced strains which differed from each other on the second chromosome but shared the same genetic background. We used isofemale lines obtained from two collections made in Serra do Japi, SP, Brazil (23°11'S, 46°40'W) in July 1994 and May 1995. The females were crossed with marker strains (CR26A, CR26B, CR26C and CR26J) and the second chromosome then made autozygous by inbreeding (figure 1).

Females from the isofemale line were initially crossed with males from strain CR26C. From the offspring, we selected males with phenotype Impar, which were heterozygous for the recessive markers *mt* (on the second chromosome), *cr* (on the fourth chromosome) and *al* (on the fifth chromosome) and for the dominant marker *Im* (on the third chromosome). These males were crossed with females from strain CR26A and from male offspring with the phenotypes Impar, coral and alfinete were selected. Thus, the fourth and fifth chromosomes from the isofemale line were replaced by the corresponding marked chromosomes. The selected males were crossed with females CR26A and the larvae from these crosses were karyotyped. If the karyotype was *DA-PA0*, males with the phenotypes Impar, coral and alfinete were crossed with females from strain CR26B. If the karyotype was *DV-PC0*, *DS-PC0* or *DP-PC0*, males with the same phenotype were crossed with females from strain CR26J. In both cases, delta, alfinete and coral males and females of the offspring would form the next cross. The flies produced in this cross that were only coral and alfinete were used to start the new strain. If the second chromosome from the isofemale line was lethal, males and females delta were crossed and the strain was kept balanced.

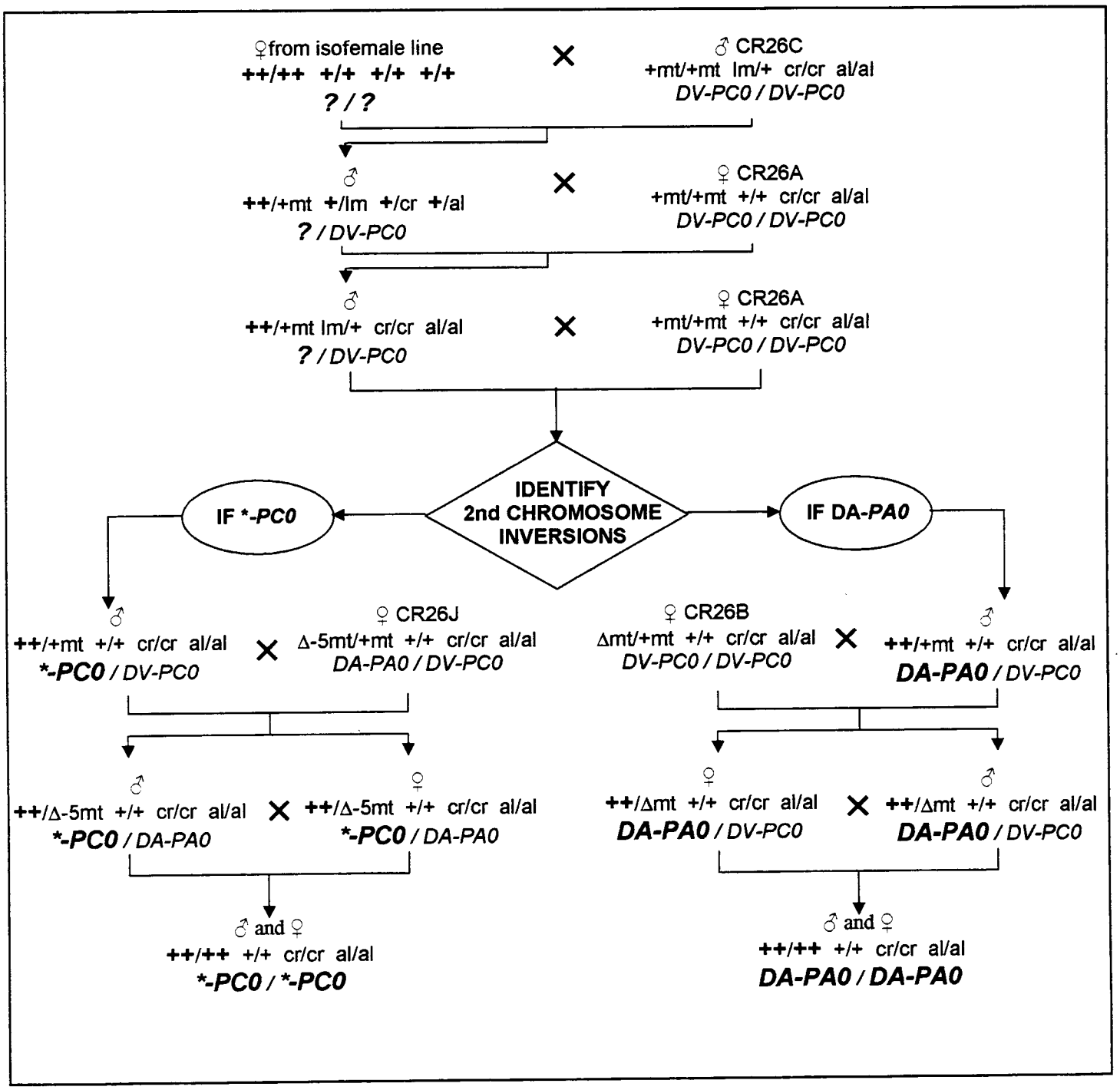

Figure 1: Crosses made to obtain strains with different second chromosomes on the same genetic background. All original chromosomes from the isofemale line were replaced, except for the second chromosome, which was made autozygous. The chromosomes from the isofemale line are in bold.

Using this approach, we obtained strains that were differentiated in the second chromosome, but not in the other chromosomes, *i.e.* they shared the same genetic background. Overall, 25 strains (14 *PA0* and 13 *PC0*) were produced.

First instar larvae were collected from each strain and 15 larvae per vial were kept at 20°C with culture medium (16% yeast, 2% sugar and 2.5% agar) until all adults had emerged.

The patterns of abdominal spots were analyzed in only 10 strains for each karyotype since the second chromosome of the remaining strains carried at least one lethal gene, which made it impossible to examine individuals autozygous for this chromosome. All strains carrying *PA0* were *DA* in the distal region of the chromosome, whereas strains carrying *PC0* also carried o *DV, DS* or *DP* in this region. It was not possible to run the experiment with all strains simultaneously, because there were 20 strains and only one person was responsible for classifying the phenotypes of the flies. Thus, sets of up to six strains were examined simultaneously until all strains were analysed.

The statistical analysis was done using ANOVA of the means of the number of spots per individual for each sex of each strain, to allow assessment of the the effects of the second chromosome karyotype, sex and the interaction between these two factors. The advantage of using the mean of each strain instead of the number of spots for each individual is that as sample size increases the means approach the normal distribution (Sokal, 1995).

Crosses between strains with the same genetic background.

Since autozygosis for an entire chromosome is quite unlikely in nature, we planned crosses between strains with the same karyotype, so that the resulting offspring would simultaneously be homokaryotypic and alozygous for the proximal inversions of the second chromosome. The crosses were chosen randomly among all possibilities, with the condition that each strain would participate in only two crosses. In this way, the number of crosses was equal to the number of strains available.

Since it was again not possible to make all of the crosses at once, they were done in sets of five. One of these crosses was repeated each time a new set of crosses was made.

In order to ensure that there were no changes in the environmental conditions during the experiment.

Crosses were made with males from one strain and females from another strain. Reciprocal crosses were not done. Again, first instar larvae were collected from the offspring of each cross and placed in vials containing 10 ml of culture medium. These larvae were kept under four conditions: 12 or 96 larvae per vial, at 20°C, and 12 or 96 larvae per vial, at 16.5°C.

The effect of each of these variables (karyotype, temperature, density and sex) on the color polymorphism was tested by ANOVA, in a manner analogous to the analysis of the strains.

RESULTS

Genetic analysis

Effect of the sex chromosomes

Table 1 shows that there were no significant differences between the results of the reciprocal crosses between strains NA (pure 3) and ITC-29I (pure 0). Therefore sex chromosomes had no significant effect.

Table 1: Numbers of flies from each phenotype in reciprocal crosses 1 (females NA and males ITC-29I) and 2 (females ITC29-I and males NA) raised at 16.5°C (Exact Fisher's Test, P=0.15 and P=0.49 for females and males, respectively).

		Number of spots				
Cross	**Sex**	**0**	**1**	**2**	**3**	**Total**
1	males	0	60	36	8	104
	females	0	127	1	0	128
2	males	0	42	31	3	76
	females	0	83	2	2	87

Autosomes

The ANOVA of the results of the genetic analysis (table 2) revealed that the second chromosome was the most important chromosome in determining of the color polymorphism. The ANOVA on table 2 also revealed significant effects of sex and the fifth chromosome; the third chromosome exerted an effect that bordered on significance (P = 0,088). Figure 2 shows that the phenotypes changed considerably, depending on the origin of the second chromosome (compare the lower and upper halves of each graph in the figure). In addition, ANOVA detected no significant effects for any of the interactions (not shown).

Table 2: ANOVA for the data used to assess the effects of each chromosome and sex on the number of abdominal spots. Significant values of P are in bold.

Source	**Sum-of-squares**	**d.f.**	**Mean square**	**F-ratio**	**P**
II	64.440	1	64.440	179.932	**<0.001**
III	0.514	1	0.514	1.435	0.231
IV	1.045	1	1.045	2.918	0.088
V	2.180	1	2.180	6.087	**0.014**
Sex	2.184	1	2.184	6.099	**0.014**
Error	367.087	1025	0.358		

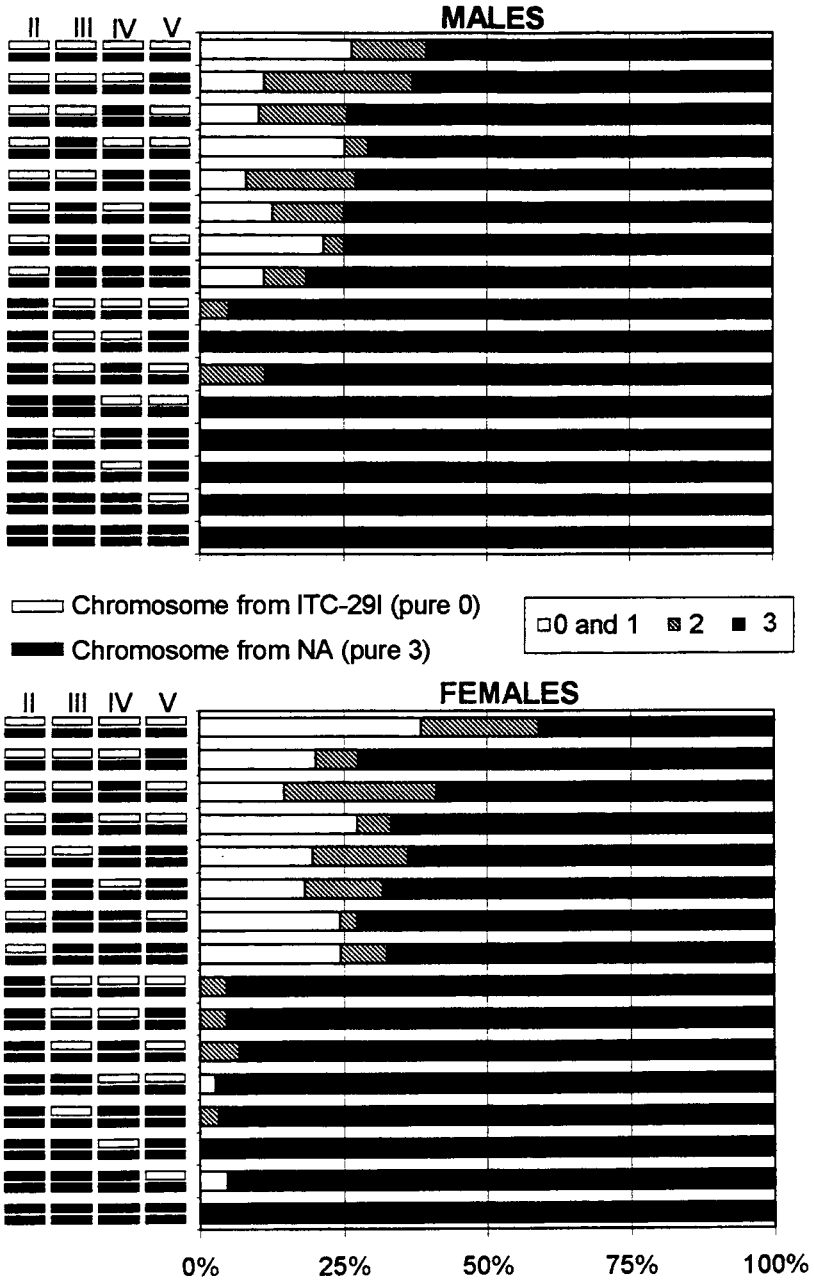

Figure 2: Percentages of the patterns of color polymorphism based on genetic analysis showing the effects of each chromosome on males and females separately. In each chart, the effect of the second chromosome becomes evident when the upper half (where the second chromosome is from a pure 0 strain) is compared with the lower half (where the second chromosome is from a pure 3 strain).

Strains with different second chromosomes on the same genetic background.

Figure 3 shows that strains in which the karyotype was *PA0* usually had fewer spots than those in which the karyotype was *PC0.* The difference between the two karyotypes was significant, as was the difference between sexes. There was no significant interaction between these two factors (table 3).

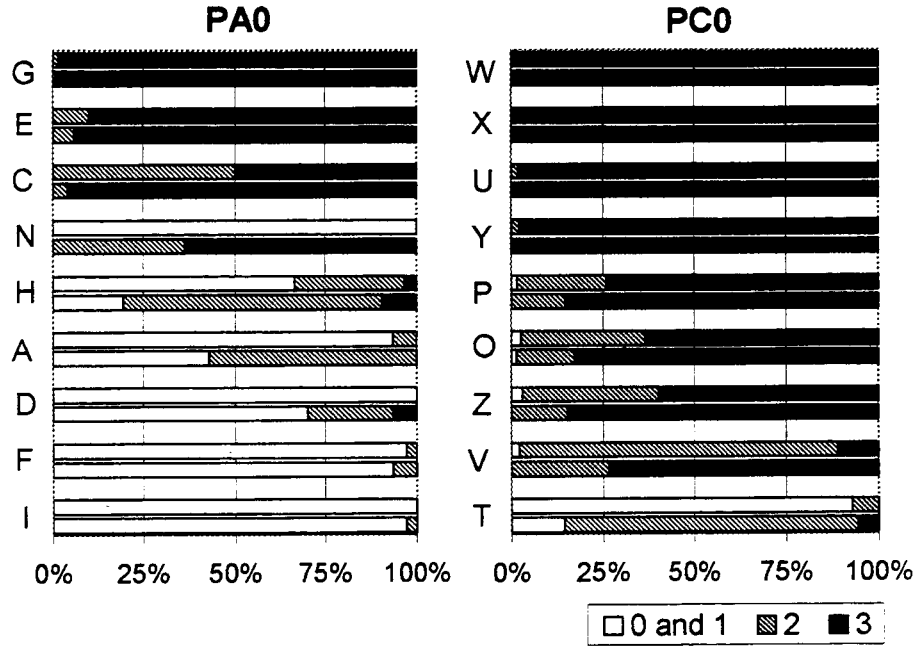

Figure 3: Percentages of the patterns of color polymorphism in strains with different second chromosomes on the same genetic background. In each pair of bars, the upper bar indicates the proportions of the phenotypes among the males and the lower bar refers to females

Table 3: Results of the ANOVA applied to the average number of spots per individual of each strain to assess the effects of karyotype, sex, strain and interaction between genotype and sex. Significant values of P are in bold.

Source	Sum-of-squares	d.f.	Mean square	F-ratio	P
Karyotype (K)	6.167	1	6.167	68.743	**<0.001**
Sex (S)	0.938	1	0.938	10.458	**0.005**
Strain	14.546	16	0.909	10.135	**<0.001**
K x S	0.064	1	0.064	0.710	0.412
Error	1.435	16	0.090		

Crosses

ANOVA (Table 4) shows that the karyotype of the second chromosome had a significant effect on the phenotype of the color polymorphism among the offspring of crosses between strains with the same genetic background for this chromosome. It can be seen in figure 4 that crosses between strains carrying *PA0* tended to produce offspring with fewer abdominal spots than crosses between strains carrying PC0. Thus, there is a non-random association between karyotype and color polymorphism.

Table 4 also shows that temperature had a significant effect and was the source of most of the variation, leading to flies with more spots at lower temperatures. The effect of sex was also significant so that males tended to have more spots than females (tables 5 and 6). In addition, there were significant interactions between karyotype and temperature, and between sex and temperature (table 4).

Table 4: Results of the ANOVA applied to the average number of spots in each cross, to assess the effects of karyotype, density, temperature, sex, cross (nested by karyotype) and all possible interactions. Significant values of P are in bold.

Source	Sum-of-squares	d.f.	Mean square	F-ratio	P
Karyotype (K)	6.388	1	6.388	53.725	**<0.001**
Density (D)	0.026	1	0.026	0.215	0.644
Temperature (T)	34.804	1	34.804	292.709	**<0.001**
Sex (S)	2.587	1	2.587	21.753	**<0.001**
Cross [karyotype]	21.609	23	0.940	7.902	**<0.001**
K × D	0.067	1	0.067	0.563	0.454
K × T	2.856	1	2.856	24.017	**<0.001**
K × S	0.031	1	0.031	0.257	0.613
D × T	0.018	1	0.018	0.148	0.701
D × S	0.001	1	0.001	0.008	0.931
T × S	0.968	1	0.968	8.142	**0.005**
K × D × T	0.010	1	0.010	0.085	0.770
K × D × S	0.003	1	0.003	0.024	0.876
K × T × S	0.049	1	0.049	0.412	0.522
D × T × S	0.002	1	0.002	0.017	0.897
K × D × T × S	0.007	1	0.007	0.060	0.808
Error	18.906	159	0.119		

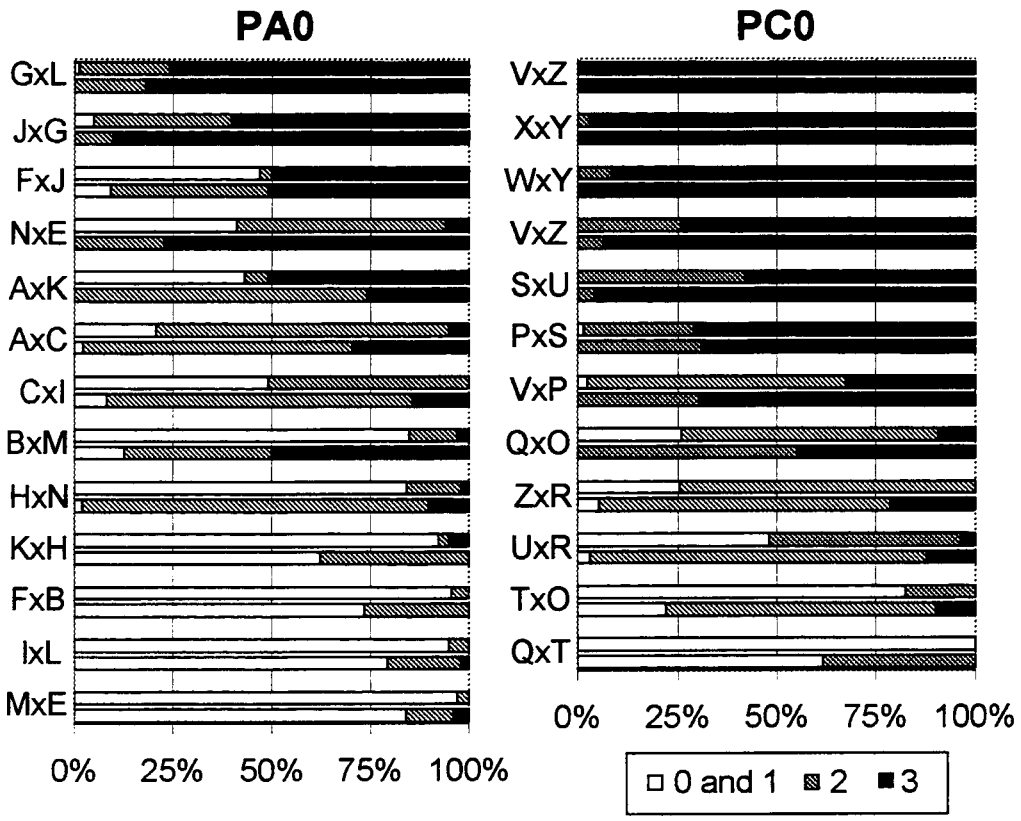

Figure 4: Patterns of color polymorphism for crosses with different second chromosomes on the same genetic background. The data are for flies raised in 20°C at density of 12 larvae per vial. In each pair of bars, the upper bar indicates the proportions of the phenotypes among the males and the lower bar refers to females

Table 5: Average number of spots per offspring in crosses between strains *PA0*, in relation to temperature and larval density.

		16.5°C				20°C			
		12 larvae		96 larvae		12 larvae		96 larvae	
Cross	**Sex**	**n**	**Average**	**n**	**Average**	**N**	**Average**	**n**	**Average**
I x L	m	22	3	11	3	48	1.23	42	1.17
I x L	f	16	3	9	3	38	1.03	29	1.14
H x N	m	24	3	29	3	49	2.08	30	2.00
H x N	f	28	3	14	2.86	51	1.18	24	1.25
F x B	m	10	3	13	2.92	30	1.27	5	1.20
F x B	f	6	2.67	14	2.71	44	1.02	6	0.83
C x I	m	27	3	9	3	49	2.06	29	1.93
C x I	f	35	3	15	3	43	1.51	40	1.38
J x G	m	26	3	11	3	41	2.90	21	3.00
J x G	f	16	3	17	3	63	2.56	34	2.59
A x C	m	23	3	9	3	44	2.27	18	2.50
A x C	f	33	3	22	3	39	1.85	20	1.95
K x H	m	12	3	16	3	45	1.38	31	1.81
K x H	f	19	2.68	32	2.63	39	1.05	42	1.00
M x E	m	25	2.64	25	2.80	25	1.20	34	1.24
M x E	f	19	2.68	21	2.57	33	1.03	20	1.00
N x E	m	30	3	4	2.75	22	2.77	10	2.50
N x E	f	26	2.92	17	3	17	1.65	13	2.00
F x J	m	28	2.93	134	2.76	88	2.42	26	1.69
F x J	f	35	2.69	66	2.23	92	2.03	30	1.20
G x L	m	30	3	90	3	50	2.82	35	2.91
G x L	f	46	2.96	60	3	75	2.75	42	2.88
B x M	m	20	2.30	62	2.34	56	2.38	18	2.06
B x M	f	31	2.58	70	2.34	33	1.18	38	1.74
A x K	m	25	2.72	40	2.68	31	2.26	41	1.88
A x K	f	26	2.73	62	2.52	86	2.08	46	1.07
Average (males)			2.89 ± 0.06		2.87 ± 0.06		2.08 ± 0.17		1.99 ± 0.17
Average (females)			2.84 ± 0.05		2.76 ± 0.08		1.61 ± 0.17		1.54 ± 0.18

Table 6: Average number of spots offspring for crosses between strains *PC0*, in relation to temperature and larval density.

		16,5°C				20°C			
		12 larvae		96 larvae		12 larvae		96 larvae	
Cross	**Sex**	**n**	**Average**	**n**	**Average**	**n**	**Average**	**n**	**Average**
W x Y	m	36	3	45	3	53	3	47	3
W x Y	f	33	3	56	3	60	2.92	43	2.91
X x Y	m	16	3	-	-	18	3	21	2.86
X x Y	f	23	3	-	-	32	2.97	39	3
W x X	m	35	3	30	3	61	3	38	3
W x X	f	27	3	38	3	77	3	50	3
Q x T	m	28	2.93	32	2.90	47	1.38	35	1.46
Q x T	f	34	2.36	30	2.53	46	1	29	1.03
P x S	m	30	3	25	3	84	2.69	29	2.69
P x S	f	28	3	51	3	73	2.70	30	2.13
S x U	m	25	3	26	3	48	2.96	35	2.94
S x U	f	38	3	25	3	50	2.58	39	2.44
T x O	m	53	2.89	15	3	50	1.88	37	2.05
T x O	f	46	2.59	15	2.73	51	1.17	42	1.33
V x P	m	36	3	34	3	33	2.70	35	2.20
V x P	f	39	3	45	3	43	2.30	35	2.09
U x R	m	39	3	28	3	33	2.09	44	2.11
U x R	f	42	2.98	56	2.95	54	1.56	46	1.28
Z x R	m	59	3	28	3	56	2.16	32	2.19
Z x R	f	61	2.75	34	2.79	51	1.75	22	2
Q x O	m	50	3	26	2.92	58	2.45	26	2.54
Q x O	f	64	2.89	31	2.87	54	1.83	31	2.13
V x Z	m	39	3	28	3	62	2.94	43	3
V x Z	f	46	3	40	3	66	2.74	38	2.92
Average (males)			2.98 ± 0.01		2.98 ± 0.01		2.52 ± 0.15		2.50 ± 0.14
Average (females)			2.88 ± 0.06		2.90 ± 0.04		2.21 ± 0.21		2.19 ± 0.20

Control cross

The phenotype of the cross used as the control was particularly favorable because it was intermediate at 20°C. This cross produced offspring in which none of the extreme phenotypes (0 and 3) was the commonest, thus allowing us to detect any displacement of the phenotype by environmental changes.

The use of a control cross ensured that environmental variables did not change during the course of the experiment. However, since significant differences between the offspring of control crosses done on different dates were observed (ANOVA, not shown), the crosses were reanalyzed, using the logarithm of the ratios between the mean of each cross and the mean of the corresponding control cross, considering the date, sex, density and temperature. In this way, the means of the crosses were standardized to the control, in order to eliminate all environmental effects, including the influence of the factors

mentioned above. This second analysis (not shown) reduced the effects of temperature and density, which became non-significant whereas the effect of sex remained. The interactions between karyotype and temperature, and sex and temperature also persisted and a new marginally significant (P=0.049) interaction (between density and sex) appeared.

The effect of karyotype remained highly significant and became the factor with the highest influence on the character (F=48.52; P=0,001). Thus, the mathematical correction diminished the environmental effects and emphasized the difference between the two karyotypes.

DISCUSSION

Genetic analysis

Chromosomal analysis is useful for showing the genetic variation of a character among chromosomes. This classic approach has been used in the genetic analysis of hybrid sterility between *D. pseudoobscura* and *D. persimilis* (Dobzhansky, 1936) and in the genetic analysis of the DDT resistance in *D. melanogaster* (Crow ,1957). Both authors concluded that the inheritance of the character was polygenic, with at least one locus on each chromosome. An almost identical genetic analysis of *D. mediopunctata* to that used here in this work revealed the existence of several autosomic suppressors of *sex-ratio*, with at least one locus in each major autosome (Carvalho and Klaczko, 1993).

As shown here, the color polymorphism in *D. mediopunctata* was determined by genes localized in the second and fifth chromosomes. The influence of the second chromosome was much higher than for any of the other chromosomes, suggesting the existence of a major locus or several minor effect loci on this chromosome.

Caveat

It is important to bear in mind that this type of genetic analysis only detects differences between strains. Thus, whilst other chromosomes may contribute to the determination of this character, the strains used in our experiments carried the same alleles (or alleles for equivalent effects) on these loci. In this case, these loci would not be detected

in our genetic analysis. This possibility was minimized by the choice of strains selected for the maximum difference between them.

Temperature, sex and density

The effect of temperature was very intense since it increased the number of spots at the lower temperature and was responsible for most of the variation in the results of crosses between strains with the same background. In *D. melanogaster* and *D. simulans*, Gibert *et al.* (1996) also found a strong temperature effect on abdominal pigmentation, similar to that which we observed in *D. mediopunctata*. In *D. melanogaster* and *D. simulans*, the influence of temperature was similar on the trident pigmentation on the thorax (Capy *et al.*, 1988). In *D. simulans*, the trident is evident only in flies raised at low temperatures. Although there is little phenotypic plasticity for pigmentation in *D. kikkawai*, a more intense pigmentation is seen in flies raised at lower temperatures (Gibert *et al.*, 1999).

The effect of sex was always significant with males tending to have more spots than females. These results are in agreement with those of Frota-Pessoa (1954), who observed that *D. mediopunctata* males usually had more spots, which also tended to be darker than in females. A significant interaction between sex and temperature was observed, suggesting that males and females have different reaction norms.

All of these results agree with our previous findings for this species (Hatadani, Basso da Silva, Valente and Klaczko, *submitted ms.*).

Chromosome inversions

Since the second chromosome, which is the most polymorphic in *D. mediopunctata* (Peixoto and Klaczko, 1991; Ananina et al., 2002), showed greater influence on the character, we examined the correlations between inversions and the number of abdominal spots, *i.e.* whether different second chromosome karyotypes had different effects on the phenotype color polymorphism.

Crossing-over within the inverted region in inversion heterozygotes results in unviable gametes so that no recombinants between genes contained in the inversion are

produced. Dobzhansky (1970) believed that the suppression of recombination would be advantageous if the inverted region carried a supergene, such that inversions which kept coadapted gene complexes together would be favored by natural selection. Evidence for polymorphism conditioned by supergenes has been found in land snails (Murray and Clarke, 1976; Jones *et al.*, 1977) and butterflies (Clarke and Sheppard, 1962; Mallet, 1989; Mallet *et. al.*, 1990; Gordon and Das, 1998).

Since chromosome inversions can behave exactly like supergenes, as predicted by Ford (1943), the existence of polymorphisms would be expected to be determined by different chromosome inversions. Several studies have demonstrated the influence of *Drosophila* chromosome inversions on morphological characters such as wing size and shape in *D. mediopunctata* (Bitner-Mathé *et al.*, 1995), extra bristles in *D. melanogaster* (Garcia-Vázquez *et al.*, 1989; Izquierdo *et al.*, 1991) and *D. ananassae* (Das *et al.*, 1994), and size-related traits in *D. buzzati* (Hasson *et al.*, 1992; Norry *et al.*, 1995, 1997; Bertrán *et al.*, 1998). Our results show that the color polymorphism in *D. mediopunctata* is associated with chromosome inversions. Karyotype *PA0* was associated with phenotypes with fewer abdominal spots, whereas *PC0* was associated with phenotypes with more spots.

Because of the strong linkage disequilibrium between proximal and distal inversions on the second chromosome, *PA0* was mainly linked to *DA* whereas *PC0* was mainly linked to *DP, DV* and *DS* (Peixoto and Klaczko, 1991). Because of this, the strains analyzed in this work were *DA-PA0, DV-PC0, DS-PC0* and *DP-PC0*. Hence, it is possible that genes determining the color polymorphism are also located in on the distal region of this chromosome, and that the conclusions drawn here can be extended to the inversions in the distal region.

There was a significant interaction between karyotype and temperature, suggesting that the gene complexes within each karyotype had different reaction norms related to temperature. However, this interaction could simply reflect the limits of this character's variation. The results showed that for the same decrease in temperature, the offspring of crosses between strains *PA0* had a greater increase in the number of spots than did offspring from crosses between strains *PC0*. However, *PC0* is already associated with more spots, even at 20°C (high temperature) and is therefore closer to the upper limit of the possible average number of spots (three), whereas as temperature decreases, the average number of spots can increase only up to this limit, resulting in the interaction.

Our results agree with two other data sets already found for this species. First, inversion frequencies in *D. mediopunctata* change during the year so that *PA0* tend to be more frequent on colder months than in warmer months, whereas *PC0* undergoes opposite changes (Klaczko, 1995). There is a significant negative correlation between the frequency of PA0 and temperature on collection site, while PC0 has an opposite pattern (Ananina *et al., personal communication*)

In addition, there is a positive correlation between the frequence of *PA0* and altitude whereas this correlation is negative for *PC0* (Klaczko, 1995). These data suggest that *PA0* is more adapted to lower temperatures and *PC0* to higher temperatures. Second, the color polymorphism in *D. mediopunctata* seems to be under natural selection which occurs in the opposite direction to the temperature influence, i.e., when there is a decrease in the temperature, selection favors genotypes for fewer spots and vice versa (Hatadani, Basso da Silva, Valente and Klaczko, *submitted*). Since *PA0* determines phenotypes with fewer spots and this karyotype increases in frequency in colder months, an increase in the frequency of genotypes leading to phenotypes with fewer spots is also expected in colder months. Thus, the patterns of variation in the inversions and color polymorphism are compatible and in good correspondence. Since *PA0* is related to lower temperatures, and *PC0* to higher temperatures, it is possible that these inversions involve the accumulation of genes adapted for each of these temperature conditions. So, if the genes that determine abdominal color pattern are located in these inversions, natural selection would have favored an association in which the linked alleles would be more adapted to the temperatures in which each karyotype is more frequent.

ACKNOWLEDGEMENTS

It is a pleasure to thank Laércio Santana for technical help; and Stephen Hyslop for correcting the English version. Profs. Jean David and Brigitte Moreteau gave insightful suggestions. Supported by grants from: Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq); Coordenação de Aperfeiçoamento de Pessoal de Ensino Superior (CAPES); Fundação de Amparo à Pesquisa de São Paulo (FAPESP).

REFERENCES

ANANINA, G. et al. Polytene chromosome map and inversion polymorphism in *Drosophila mediopunctata.* **Mem. Inst. Oswaldo Cruz**, Rio de Janeiro, n. 97, p. 691-694. 2002.

BERTRÁN, E.; SANTOS, M.; RUIZ, A. Antagonistic pleiotropic effect of second-chromosome inversions on body size and early life-history traits in *Drosophila buzzatii.* **Evolution**, n. 52, p. 144-154. 1998.

BITNER-MATHÉ, B. C.; PEIXOTO, A.; KLACZKO, L. B. Morphological variation in a natural population of *Drosophila mediopunctata*: altitudinal cline, temporal changes and influence of chromosome inversions. **Heredity**, n. 75, p. 54-61. 1995.

CAPY, P.; DAVID J. R.; ROBERTSON, A. Thoracic trident pigmentation in natural populations of Drosophila simulans: a comparison with *D. melanogaster*. **Heredity**, n. 61, p. 263-268. 1988.

CARVALHO, A. B.; KLACZKO, L. B. Autosomal suppressors of sex-ratio in *Drosophila mediopunctata.* **Heredity**, n. 71, p. 546-551. 1993.

CROW, J. F. Genetics of insect resistance to chemicals. **Annu. Rev. Entomol.**, n. 2, p. 227-246. 1957.

CLARKE, C. A.; SHEPPARD, P. M. Supergenes and mimicry. **Heredity**, n. 14, p. 175-185. 1960.

DA CUNHA, A. B. Genetic analysis of the polymorphism of color pattern in *Drosophila polymorpha.* **Evolution**, n. 3, p. 239-251. 1949.

DAHLGAARD, J.; HASSON, E.; LOESCHCKE, V. Behavioral differentiation in oviposition activity in *Drosophila buzzatii* from highland and lowland populations in Argentina: plasticity or thermal adaptation? **Evolution**, n. 55, p. 738-747. 2001.

DAS, A.; MOHANTY, S.; PARIDA; B. B. Inversion polymorphism and extra bristles in Indian populations of *Drosophila ananassae*: joint variation. **Heredity**, n. 73, p. 405-409. 1994.

DOBZHANSKY, T. Genetics of natural populations. XIV. A response of certain gene arrangements in the third chromosome of *Drosophila pseudoobscura* to natural selection. **Genetics**, n. 32, p. 142-160. 1947.

DOBZHANSKY, T. **Genetics of the evolutionary process.** New York:Columbia University Press. 1970.

FORD, E. B. **Ecological Genetics.** London: Chapman & Hall. 1964.

FROTA-PESSOA, O. Revision of the *tripunctata* group of *Drosophila* with description of fifteen new species (Drosophilidae, Diptera). **Arq. Mus. Para.**, n. 10, p. 253-304. 1954.

GARCÍA-VÁZQUEZ, E.; SÁNCHEZ-REFUSTA, F.; RUBIO, J. Chromosome inversions and frequency of extra bristles in natural populations of *Drosophila melanogaster. Journal of* **Heredity**, n. 80, p. 193-196. 1989.

GIBERT, P. et al. Growth temperature and adult convergence of reaction norms in sympatric populations? **Evolution**, n. 50, p. 2346-2353. 1996.

GIBERT, P. et al. Phenotypic plasticity of abdominal pigmentation in *Drosophila kikkawai*: multiple interactions between a major gene, sex, abdomen segment and growth temperature. **Genetica**, n. 105, p. 165-176. 1999.

GIBSON, A. R.; FALLS, J. B. Thermal biology of the common garter snake *Thamnophis sirtalis* (L.) II. The effects of melanism. **Oecologia**, n. 43, p. 99-109. 1979.

GORDON, I. J.; SMITH, D. A. S. Body size and colour-pattern genetics in the polymorphic mimetic butterfly *Hypolomnas misippus* (L.). **Heredity**, n. 80, p. 62-69. 1998.

HASSON, E. et al. The evolutionary history of *Drosophila buzzatii.* XXIV. Second chromosome inversions have different average effects on thorax length. **Heredity**, n. 68, p. 557-563. 1992.

HEED, W. B.; BLAKE, P. R. A new color allele at the *e* locus of *Drosophila polymorpha* from northern South America. **Genetics**, n. 48, p. 217-234. 1963.

IZQUIERDO, J. I.; GARCÍA-VÁZQUEZ, E.; VILLAR, B. Correlated variation of chromosomal inversion (3R)C and extra bristles in *Drosophila melanogaster*. **Heredity**, n. 67, p. 183-187. 1991.

JONES, J. S.; LEITH, B. H.; RAWLINGS, P. Polymorphism in *Cepaea*: a problem with too many solutions? **Annu. Rev. Ecol. Syst.**, n. 8, p. 109-143. 1977.

KLACZKO, L.B. In: LEVINE,L. (Ed.) **Genetics of Natural Populations: the Continuing importance of Theodosius Dobzhansky**. Pp. 140-153. New York: Columbia University Press. 1995.

MALLET, J. The genetics of warning colour in Peruvian hybrid zones of *Heliconius erato* and *Heliconius melpomene*. **Proc. R. Soc. Lond. B**, n. 236, p. 163-185. 1989.

MALLET, J. et al. Estimates of selection and gene flow from measures of cline width and linkage disequilibrium in *Heliconius* hybrid zones. **Genetics**, n. 124, p. 921-986. 1990.

MARTINEZ, M. N.; CORDEIRO, A. R. Modifiers of color pattern genes in *Drosophila polymorpha*. **Genetics**, n. 64, p. 573-587. 1970.

MURRAY, J.; CLARKE, B. Supergenes in polymorphic land snails. I. *Partula taeniata*. **Heredity**, n. 37, p. 271-282. 1976.

NORRY, F. M. et al. An adaptive chromosomal polymorphism affecting size-related traits, and longevity selection in a natural population of *Drosophila buzzatii*. **Genetica**, n. 96, p. 285-291. 1995.

NORRY, F. M.; VILARDI, J. C.; HASSON, E. Correlations among size-related traits are affected by chromosome inversions in an adaptive polymorphism in *Drosophila buzzatii*. **Heredity**, n. 79, p. 585-590. 1997.

PEIXOTO, A. A.; KLACZKO, L. B. Linkage disequilibrium analysis of chromosomal inversion polymorphisms of Drosophila. **Genetics**, n. 129, p. 773-777. 1991.

PIPKIN, S.B. Mesonotal color polymorphism in *Drosophila l. lebanonensis*. **Genetics**, n. 47, p. 1275-1290. 1962.

POWELL, J. R. **Progress and Prospects in Evolutionary Biology. The Drosophila Model**. New York: Oxford University Press. 1997.

QUINTANA, A.; PREVOSTI, A. Genetic and environmental factors in the resistance of *Drosophila subobscura* adults to high temperature shock. III. Chromosomal-inversion and enzymatic polymorphism variation in lines selected for heat shock resistance. **Genetica**, n. 84, p. 165-170. 1991.

RODRÍGUEZ, C.; FANARA, J. J.; HASSON, E. Inversion polymorphism, longevity and body size in a natural population of *Drosophila buzzatii*. **Evolution**, n. 53, p. 612-620. 1999.

RUIZ, A. et al. The evolutionary history of *Drosophila buzzatii*. VIII. Evidence for endocyclic selection acting on the inversion polymorphism in a natural population. **Evolution**, n. 40, p. 740-755. 1986.

SAAVEDRA, C. C. R. et al. A descriptive and analytical study of our neotropical drosophilid communities. **J. Zool. Syst. Evol. Res.**, n. 33, p. 62-74. 1995.

SENE, F. M. et al. Preliminary data on the geographical distribution of *Drosophila* species within morphoclimatic domains of Brazil. **Papéis Avuls. Zoo. S. Paulo**, n. 33, p. 315-326. 1980.

SOKAL, R. R.; ROHLF, F. J. 1995. **Biometry: the principles and practice of statistics in biological research**. New York.: W. H. Freeman & Co.

WRIGHT, S.; DOBZHANSKY, T. 1946. Genetics of natural populations. XII. Experimental reproduction of some of the changes caused by natural selection in certain populations of *Drosophila pseudoobscura*. **Genetics**, n. 31, p. 125-156.

CONCLUSÕES GERAIS

O efeito da temperatura

O efeito da temperatura sobre o polimorfismo de *Drosophila mediopunctata* é bastante intenso, de modo que é o fator responsável pela maior proporção da variação nos experimentos realizados neste trabalho. Animais que se desenvolveram sob temperaturas mais altas têm menos manchas abdominais. Esta observação está de acordo com o que já foi observado em outras espécies de *Drosophila*. A explicação adaptativa mais aceita é a de que fenótipos de pigmentação mais intensa são mais vantajosos quando as temperaturas são mais baixas porque favorecem a absorção de calor e, conseqüentemente o metabolismo do animal.

O efeito da densidade

A densidade não apresentou efeito significativo. Isso pode significar que a norma de reação desse caráter não muda muito de acordo com a densidade em que se desenvolvem os animais. Ou ainda que se há seleção natural dependente de densidade atuando sobre os animais analisados nestes experimentos, ela é fraca.

O efeito do sexo

O efeito do sexo foi quase sempre significativo: os machos tendem a ter mais manchas que as fêmeas. Machos e fêmeas também são diferentes entre si pela norma de reação do caráter: fêmeas são mais sensíveis à variação de temperatura que os machos.

Interação genótipo-ambiente

A interação entre cariótipo e temperatura indica a existência de interação genótipo-ambiente para esse caráter, ou seja, que diferentes genótipos possuem diferentes normas de reação.

Variação fenotípica no campo

Houve diferença significativa entre as duas coletas realizadas em Campinas (SP), assim como nas coletas de Porto Alegre (RS). Essa diferença pode ser atribuída principalmente à variação ambiental ocorrida entre as duas datas de coleta, mas também foram detectadas diferenças de origem genética. É provável que a variação de origem ambiental entre coletas seja devida à diferença de temperatura local entre as datas de coleta.

Variação fenotípica no laboratório

Moscas criadas sob condições padronizadas no laboratório fornecem uma medida da variação genética que pode ou não ter ocorrido entre as duas coletas de cada localidade. Observou-se que a variação genética ocorre em sentido contrário à variação causada pela influência da temperatura, ocasionando a formação de um padrão de variação contragradiente e sugerindo a ação de seleção natural. Esta seleção natural não está de acordo com o que já foi observado em outras espécies de *Drosophila*, em que moscas geneticamente mais escuras foram encontradas em localidades mais frias, isto é, a variação genética reforça o efeito da variação ambiental. Isto quer dizer que a termorregulação não é a única função adaptativa da pigmentação em *D. mediopunctata*.

Análise genética

A análise genética revelou que existem genes que determinam o padrão de coloração nos cromossomos II e V. O efeito do cromossomo II é muito maior que o do cromossomo V, o que sugere que haja neste cromossomo um gene principal que determine o caráter ou vários genes ligados de menor efeito.

Associação não-aleatória entre os padrões de polimorfismo de coloração e inversões do cromossomo II.

Dentre as estirpes analisadas, foi possível verificar a existência da associação entre os cariótipos da inversão proximal do cromossomo II e os padrões de manchas abdominais: PA0 está associada a fenótipos de menos manchas e PC0 está associado a fenótipos de

maior número de manchas. Isso significa que as inversões do cromossomo II nesta espécie podem estar agindo como um supergene, acumulando alelos coadaptados.

Síntese

Considerando as seguintes informações:

a. PA0 aumenta de freqüência quando as temperaturas são mais baixas, enquanto PC0 aumenta de freqüência quando as temperaturas são mais altas;

b. PA0 está associada a fenótipos de menos manchas, enquanto PC0 está associada a fenótipos de mais manchas;

c. Indivíduos que se desenvolvem a temperaturas mais baixas tendem a ter mais manchas;

d. Quando a temperatura é mais baixa, a seleção natural favorece genótipos que condicionam menos manchas;

pode-se propor a seguinte hipótese: a associação entre PA0 e fenótipos de menos manchas seria favorecida pela seleção natural, porque a freqüência de PA0 aumenta em temperaturas mais baixas. Da mesma forma, a associação entre PC0 e fenótipos de mais manchas seria favorecida, porque a freqüência de PC0 aumenta em temperaturas mais altas. Assim, é possível explicar todos os dados obtidos neste trabalho, à luz do conhecimento prévio sobre as inversões em *D. mediopunctata,* de uma maneira coerente, concisa e parcimoniosa. Além disso, uma vez que está bem estabelecido o caráter adaptativo dessas inversões cromossômicas em relação à temperatura, essa hipótese pode ser testada por experimentos que verificassem se fenótipos de manchas abdominais cuja variação fosse independente das inversões cromossômicas (por exemplo, se fossem considerados apenas indivíduos portadores da mesma inversão) apresentam variação contrária ao sentido imposto pela temperatura. Dessa forma, o caráter adaptativo do polimorfismo de coloração, da forma como foi proposto pela hipótese, seria avaliado de forma independente da influência das inversões.

BIBLIOGRAFIA GERAL

ANANINA, G. et al. Polytene chromosome map and inversion polymorphism in *Drosophila mediopunctata.* **Mem. Inst. Oswaldo Cruz,** Rio de Janeiro, n. 97, p. 691-694. 2002.

BASSO DA SILVA, L. ; VALENTE V. L. S.. A temporal analysis of sexual activity in a natural population of *Drosophila willistoni.* **Hereditas**, n. 133, p. 211-216. 2000.

BERTRÁN, E.; SANTOS, M.; RUIZ, A. Antagonistic pleiotropic effect of second-chromosome inversions on body size and early life-history traits in *Drosophila buzzatii.* **Evolution**, n. 52, p. 144-154. 1998.

BITNER-MATHÉ, B. C.; PEIXOTO, A.; KLACZKO, L. B. Morphological variation in a natural population of *Drosophila mediopunctata*: altitudinal cline, temporal changes and influence of chromosome inversions. **Heredity**, n. 75, p. 54-61. 1995.

CAIN, A.J.; SHEPPARD, P. M. Selection in the polymorphic land snail *Cepaea nemoralis.* **Heredity**, n. 4, *p. 275-94.* 1950.

CAIN, A.J.; SHEPPARD, P. M. Natural selection in *Cepaea.* **Genetics**, n. 39, p. 89-116. 1954.

CAPY, P.; DAVID J. R.; ROBERTSON, A. Thoracic trident pigmentation in natural populations of Drosophila simulans: a comparison with *D. melanogaster.* **Heredity**, n. 61, p. 263-268. 1988.

CARVALHO, A. B.; KLACZKO, L. B. Autosomal suppressors of sex-ratio in *Drosophila mediopunctata.* **Heredity**, n. 71, p. 546-551. 1993.

CHARLESWORTH, B. The genetics of adaptation: lessons from mimicry. **Am. Nat.** , n. 144 (5), p. 839-847. 1994.

CLARKE, C. A.; SHEPPARD, P. M.. Supergenes and mimicry. **Heredity**, n. 14: 175-185. 1960.

CONOVER, D. O.; SCHULTZ, E. T. Phenotypic similarity and the evolutionary significance of countergradient variation. **TREE**, n. 10, p. 248-252. 1995.

CROW, J. F. Genetics of insect resistance to chemicals. **Annu. Rev. Entomol.**, n. 2, p. 227-246. 1957.

DA CUNHA, A. B. Genetic analysis of the polymorphism of color pattern in *Drosophila polymorpha*. **Evolution**, n. 3, p. 239-251. 1949.

DAHLGAARD, J.; HASSON, E.; LOESCHCKE, V. Behavioral differentiation in oviposition activity in *Drosophila buzzatii* from highland and lowland populations in Argentina: plasticity or thermal adaptation? **Evolution**, n. 55, p. 738-747. 2001.

DAS, A. Abdominal pigmentation in *Drosophila melanogaster* females from natural Indian populations. **J. Zool. Sys. Evol. Res.**, n. 33, p. 84-87. 1995.

DAS, A.; MOHANTY, S.; PARIDA, B. B. Inversion polymorphism and extra bristles in Indian populations of *Drosophila ananassae*: joint variation. **Heredity**, n. 73, p. 405-409. 1994.

DOBZHANSKY, T. Genetics of natural populations. XIV. A response of certain gene arrangements in the third chromosome of *Drosophila pseudoobscura* to natural selection. **Genetics**, n. 32, p. 142-160. 1947.

DOBZHANSKY, T. **Genetics of the evolutionary process**. New York: Columbia University Press. 1970.

DOBZHANSKY, T.; PAVAN, C. Studies on Brazilian species of *Drosophila*. **Bol. Facul. Fil. Ciên. e Letr. Univ. S. Paulo**, n. 36. 1943.

FALCONER, D.S. Selection in different environments: effects on environmental sensitivity (reaction norm) and on mean performance. **Genet. Res.**, n. 56, p. 57-70. 1990.

FORD, E. B. **Ecological Genetics**. London: Chapman & Hall. 1964.

FREIRE-MAIA, N. Segregational load in *Drosophila kikkawai* I. Crossing experiments. **Genetics**, n. 50, p. 211-219. 1964.

FROTA-PESSOA, O. Revision of the *tripunctata* group of *Drosophila* with description of fifteen new species (Drosophilidae, Diptera). **Arquivos do Museu Paranaense**, n. 10, p. 253-304. 1954.

GARCÍA-VÁZQUEZ, E.; SÁNCHEZ-REFUSTA; F.; RUBIO, J. Chromosome inversions and frequency of extra bristles in natural populations of *Drosophila melanogaster*. *Journal of* **Heredity**, n. 80, p. 193-196. 1989.

GIBERT, P. et al. Growth temperature and adult convergence of reaction norms in sympatric populations? **Evolution**, n. 50, p. 2346-2353. 1996.

GIBERT P. et al. Phenotypic plasticity of abdominal pigmentation in *Drosophila kikkawai*: multiple interactions between a major gene, sex, abdomen segment and growth temperature. **Genetica**, n. 105, p.165-176. 1999.

GIBSON, A. R.; FALLS, J. B. Thermal biology of the common garter snake *Thamnophis sirtalis* (L.) II. The effects of melanism. **Oecologia**, n. 43, p. 99-109. 1979.

GORDON, I. J.; SMITH, D. A. S. Body size and colour-pattern genetics in the polymorphic mimetic butterfly *Hypolomnas misippus* (L.). **Heredity**, n. 80, p. 62-69. 1998.

GOSTELI, M. Differential flight activity among karyotypes: daily and weather-induced changes in chromosomal inversion polymorphism in natural populations of *Drosophila subobscura*. **Genetica**, n. 84, p. 129-136. 1991.

HASSON, E. et al. The evolutionary history of *Drosophila buzzatii*. XXIV. Second chromosome inversions have different average effects on thorax length. **Heredity**, n. 68, p. 557-563. 1992.

HEED, W. B.; BLAKE, P. R. A new color allele at the *e* locus of *Drosophila polymorpha* from northern South America. **Genetics**, n. 48, p. 217-234. 1963.

HOLLOCHER, H.; HATCHER, J. L.; DYRESON, E. G. Evolution of abdominal pigmentation differences across species in the Drosophila dunni subgroup. **Evolution**, n. 54, p. 2046-2056. 2000.

IZQUIERDO, J. I.; GARCÍA-VÁZQUEZ, E.; VILLAR, B. Correlated variation of chromosomal inversion (3R)C and extra bristles in *Drosophila melanogaster*. **Heredity**, n. 67, p. 183-187. 1991.

JONES, J. S.; LEITH, B. H.; RAWLINGS, P. Polymorphism in *Cepaea*: a problem with too many solutions? **Annu. Rev. Ecol. Syst.**, n. 8, p. 109-143. 1977.

KLACZKO, L.B. In: Levine,L. (Ed.) **Genetics of Natural Populations: the Continuing importance of Theodosius Dobzhansky**. Pp. 140-153. New York: Columbia University Press. 1995.

KOPP, A.; DUNCAN, I.; CARROLL, S. B. Genetic control and evolution of sexually dimorphic characters in Drosophila. **Nature**, n. 408, p. 553-562. 2000.

LEVINS, R. Thermal acclimation and heat resistance in *Drosophila.* **Am. Nat**. , n. 103, p. 483-499. 1969.

LILLYWHITE, H. B. et al. Basking behavior, sweating and thermal ecology of the indian tree frog, *Polypedates maculatus*. **J. Herpetol.,** n. 32, p. 169-175. 1998.

MALLET, J. The genetics of warning colour in Peruvian hybrid zones of *Heliconius erato* and *Heliconius melpomene*. **Proc. R. Soc. Lond. B**, n. 236, p. 163-185. 1989.

MALLET J.; JORON, M. Evolution of diversity in warning color and mimicry: polymorphisms, shifting balance and speciation. **Annu. Rev. Ecol. Syst.** , n. 30, p. 201-233. 1999.

MALLET, J. et al. Estimates of selection and gene flow from measures of cline width and linkage disequilibrium in *Heliconius* hybrid zones. **Genetics**, n. 124, p. 921-986. 1990.

MARTINEZ, M. N.; CORDEIRO, A. R. Modifiers of color pattern genes in *Drosophila polymorpha*. **Genetics**, n. 64, p. 573-587. 1970.

MORELATTO, P.C.; LEITÃO-FILHO H. F. (Eds.) 1995. **Ecologia e preservação de uma floresta tropical urbana: Reserva de Santa Genebra.** Campinas: Editora da Unicamp.

MURRAY, J.; CLARKE B. Supergenes in polymorphic land snails. I. *Partula taeniata*. **Heredity**, n. 37, p. 271-282. 1976.

NORRY, F. M. et al. 1995. An adaptive chromosomal polymorphism affecting size-related traits, and longevity selection in a natural population of *Drosophila buzzatii*. **Genetica**, n. 96, p. 285-291.

NORRY, F. M.; VILARDI, J. C.; HASSON, E. Correlations among size-related traits are affected by chromosome inversions in an adaptive polymorphism in *Drosophila buzzatii*. **Heredity**, n. 79, p. 585-590. 1997.

OSAWA, N.; NISHIDA, T. Seasonal variation in elytral colour polymorphism in *Harmonia axyridis* (the ladybird beetle): the role of non-random mating. **Heredity**, n. 69, p. 297-307. 1992.

PEIXOTO, A. A.; KLACZKO, L. B. Linkage disequilibrium analysis of chromosomal inversion polymorphisms of Drosophila. **Genetics**, n. 129, p. 773-777. 1991.

PIPKIN, S.B. Mesonotal color polymorphism in *Drosophila l. lebanonensis*. **Genetics**, n. 47, p. 1275-1290. 1962.

POWELL, J. R. **Progress and Prospects in Evolutionary Biology. The Drosophila Model**. New York: Oxford University Press. 1997.

QUINTANA, A.; PREVOSTI, A. Genetic and environmental factors in the resistance of *Drosophila subobscura* adults to high temperature shock. III. Chromosomal-inversion and enzymatic polymorphism variation in lines selected for heat shock resistance. **Genetica**, n. 84, p. 165-170. 1991.

RODRÍGUEZ, C.; FANARA, J. J.; HASSON, E. Inversion polymorphism, longevity and body size in a natural population of *Drosophila buzzatii*. **Evolution**, n. 53, p. 612-620. 1999.

RUIZ, A. et al.. The evolutionary history of *Drosophila buzzatii*. VIII. Evidence for endocyclic selection acting on the inversion polymorphism in a natural population. **Evolution**, n. 40, p. 740-755. 1986.

SAAVEDRA, C. C. R. et al. A descriptive and analytical study of our neotropical drosophilid communities. **J. Zoo. Sys. Evol. Res.** , n. 33, p. 62-74. 1995.

SAS Institute Inc. SAS/STAT 1988. SAS/STAT™ User's Guide, Release 6.03 Edition. Cary, N.C. SAS Institute Inc., 1988. 1028 pp.

SENE, F. M. et al. Preliminary data on the geographical distribution of *Drosophila* species within morphoclimatic domains of Brazil. **Papéis Avuls. Zoo. S. Paulo**, n. 33, p. 315-326. 1980.

SOKAL, R. R.; ROHLF, F. J. **Biometry: the principles and practice of statistics in biological research.** New York: W. H. Freeman and Co. 1995.

VAL, F. C.; VILELA, C. R.; MARQUES, M. D. In: ASHBURNER, M.; Carson, H.C., Thompson, Jr, J. N. (eds.), **The genetics and Biology of Drosophila**. Vol. 3a. London: Academic Press. 1981.

VILELA, C. R. On the *Drosophila tripunctata* species group. **Rev. Bras. Ent.**, n. 36, p. 197-221. 1992.

WATT, W. B. Adaptive significance of pigment polymorphisms is *Colias* butterlies I.Variation of melanin pigment in relation to thermoregulation. **Evolution**, n. 22, p. 437-458. 1968.

WILLEMSEN, R. E.; HAILEY, A. A latitudinal cline of dark plastral pigmentation in the tortoise *Testudo hermanni* in Greece. **Herpetol. J.,** n. 9, p. 125-132. 1999.

WINDIG, J. J. Trade-offs between melanization, development time and adult size in *Inachis io* and *Araschnia levana* (Lepidoptera: Nymphalidae). **Heredity**, n. 82, p. 57-68. 1999.

WRIGHT, S. **Evolution and the Genetics of Populations**. Vol 4. Chicago: The University of Chicago Press. 1978.

WRIGHT, S.; DOBZHANSKY, T. Genetics of natural populations. XII. Experimental reproduction of some of the changes caused by natural selection in certain populations of *Drosophila pseudoobscura*. **Genetics** 31, n. 125-156. 1946.