

UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL

**O elemento transponível *hobo* e suas seqüências
relacionadas no genoma de *Drosophila simulans***

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Sumário

Abreviaturas, Símbolos e Unidades	8
Resumo	10
Abstract.....	12
CAPÍTULO 1	14
INTRODUÇÃO.....	14
Elementos Transponíveis.....	14
O elemento transponível <i>hobo</i>	21
O elemento <i>hobo</i> fora do complexo <i>melanogaster</i>	28
Seqüências relacionadas a <i>hobo</i>	29
OBJETIVOS.....	31
CAPÍTULO 2	32
Mobilization of a <i>hobo</i> -related sequence in the genome of <i>Drosophila simulans</i>	32
CAPÍTULO 3	43
Dynamics of <i>hobo</i> -related sequences in natural populations of <i>Drosophila simulans</i>	43
Abstract.....	44
Introduction	46
Materials and methods.....	51
Results	56
Discussion.....	64
References	69
Figure legends	74
Figures	76
CAPÍTULO 4	81
DISCUSSÃO GERAL, CONCLUSÕES E PERSPECTIVAS	81
REFERÊNCIAS BIBLIOGRÁFICAS	89
Anexo 1	96
Anexo 2	106

Abreviaturas, Símbolos e Unidades

µg – micrograma

µL – microlitro

BLAST – Basic Local Alignment Search Tool

bp – pares de bases

DNA – ácido desoxirribonucléico

dNTP – desoxirribonucleotídeo 5'- trifosfato

Dshs – Linhagem hipermutável de *D. simulans*

Dswhm – Mutante *white* da linhagem hipermutável de *D. simulans*

EDTA – ácido etilenodiaminotetracético

IRAP – Inter-Retrotransposons Amplified Polymorphism

ITAP – Inter-Transposons Amplified Polymorphism

ITRs – Repetições Terminais Invertidas

kb – quilobase

LINE – Long Interspersed Nuclear Elements

LTR – Longas Repetições Terminais

MEGA – Molecular Evolutionary Genetics Analysis

MITE(s) – Miniature Inverted-repeat TE(s)

MYR – milhões de anos

ng – nanogramas

O/N – durante à noite

ORF – módulo aberto de leitura

pb – pares de bases

PCR – reação de polimerização em cadeia

SDS – dodecilsulfato de sódio

SINE – Short Interspersed Nuclear Elements

TE(s) – Elemento(s) Transponíveis(s)

TSDs – Duplicações do Sítio Alvo

U - unidade

Resumo

O elemento transponível *hobo* pode estar presente sob três formas no genoma de *Drosophila simulans*: como cópias autônomas completas (ou canônicas), como cópias defectivas internamente deletadas e como seqüências relacionadas a *hobo* (ou “relics”). Algumas evidências indicam que cópias completas e internamente deletadas são aquisições recentes desse genoma, enquanto os “relics” são componentes antigos, normalmente degenerados, defectivos e até recentemente considerados imóveis. O estudo desse tipo de seqüências pode ajudar a desvendar algumas questões sobre sua origem, dinâmica e seu papel na história evolutiva da família *hobo*. No presente trabalho, buscamos contribuir ao entendimento de algumas dessas questões estudando a dinâmica de uma família particular de seqüências relacionadas a *hobo* de *D. simulans*. Primeiramente, isolamos uma seqüência “relic” *hobo* envolvida no surgimento de uma mutação *white de novo* em uma linhagem hipermutável de *D. simulans*. Esta seqüência, denominada *hobo^{v-a}*, apresenta divergência típica de elemento “relic” em relação ao elemento canônico, é defectiva como outras já descritas, porém, mobilizável, pois apresentando estruturas essenciais para mobilização bem conservadas. Além disso, apresenta alta similaridade estrutural e de seqüência com um elemento “relic” de *Drosophila sechellia*, mas parece estar ausente do genoma de *Drosophila melanogaster*. A análise populacional de *hobo^{v-a}* revela que

estes elementos são bem conservados entre diferentes populações de *D. simulans*. Apresentam, ainda, polimorfismo de sítios de inserção e variabilidade no número de cópias, o que nos dá fortes indícios de atividade atual ou recente desses elementos no genoma dessas populações. Pela similaridade compartilhada com elementos MITEs em muitas de suas características estruturais e funcionais, sugerimos, apontando algumas evidências, que elementos *hobo*^{v-a} podem ser ou uma nova família de MITEs de *Drosophila* ou, mais provavelmente, estariam se encaminhando para esse destino, utilizando o elemento canônico como fonte para sua mobilização.

Abstract

The *hobo* transposable element can be present under three forms in the *Drosophila simulans* genome: as an autonomous element (also called canonical), as internally deleted defective copies, or as *hobo*-related sequences (relics). Some evidences indicated that canonical elements and internally deleted copies are recent acquisitions of this genome, while the “relics” are old components, normally degenerated, non-functional and until recently considered as probably immobile. The study of this last type of sequences can help us to understand some questions about their origin, dynamics and their role in the evolutionary history of *hobo* family. In the present work we aim to contribute to the comprehension of some of these questions, analyzing the dynamic of a particular family of *D. simulans hobo*-related sequences. Firstly, we isolated a *hobo* “relic” element which was involved in the appearance of a *de novo white* mutation in a hypermutable strain of *D. simulans*. This element, named *hobo*^{v-a}, present the typical divergence of a relic element to a canonical element, is defective like others known until now, but is mobilizable, because it possess the structures essential to its mobilization well conserved. Moreover, it presents a high similarity of sequence and structural to a *Drosophila sechellia* relic element, but it seems to be absent from *Drosophila melanogaster* genome. The populational analysis of *hobo*^{v-a} reveal that these elements are well conserved

in the different *D. simulans* populations. Also, these elements present an insertion sites polymorphism and copy number variability, giving us strong evidences of current or recent activity in these populations genomes. By the structural and functional similarity shared between *hobo*^{v-a} elements and MITEs elements, we suggest, pointing up some evidences, that *hobo*^{v-a} elements are a new *Drosophila* MITE family or they are going to this fate, using the autonomous elements as a source to their mobilizations.

CAPÍTULO 1

INTRODUÇÃO

Elementos Transponíveis

Elementos transponíveis (TEs) são seqüências de DNA incluídas na fração de DNA medianamente repetitivo do genoma que apresentam como característica peculiar a capacidade (intrínseca ou não) de mudar de posição no genoma. Devido a essa característica especial quanto ao seu comportamento, os TEs apresentam polimorfismos de sítios de inserção e uma variabilidade no número de cópias dentro e entre espécies hospedeiras (Capy *et al.*, 1998).

Desde que foram descobertas, por Bárbara McClintock na década de 1940, estas seqüências móveis têm sido encontradas nos mais diferentes organismos investigados, sendo, inclusive, parte considerável de seus genomas. Análises recentes de seqüências de DNA mostram que 40% ou mais dos genomas humano, de camundongo e de arroz são compostos de seqüências derivadas de elementos transponíveis. Em eucariotos inferiores e bactérias, esta fração é menor (1-5%), mas ainda significativa (Kidwell e

Lisch, 2000; Lander *et al.*, 2001; Waterston *et al.*, 2002; Goff *et al.*, 2002). O número de cópias de TEs estende-se desde poucas até milhares delas. No genoma humano, por exemplo, existem mais de um milhão de cópias da família *Alu* e estas seqüências têm se movimentado tão recentemente que esta porção do genoma difere em nível populacional, sendo este polimorfismo útil para inferências filogenéticas (Capy *et al.*, 1998).

Num mesmo genoma, podem coexistir dois tipos de cópias de um dado TE: os elementos ou cópias completas, também chamados de autônomos, contendo todas as seqüências necessárias para sua transposição, e aqueles deletados, também referidos como incompletos, não autônomos, defectivos ou remanescentes. Nestes últimos, a seqüência interna pode apresentar além das deleções, algumas inserções e ou, ainda, rearranjos mais complexos. Em função disto, para sua transposição, estes elementos necessitam de fatores providos por cópias completas relacionadas.

A classificação dos TEs baseia-se, principalmente, no modo como o elemento se transpõe, mas suas características estruturais e moleculares são também consideradas. Segundo estas premissas, os elementos são agrupados em classes, subclasses, superfamílias, famílias e subfamílias. Os TEs têm sido divididos em duas grandes classes de acordo com o mecanismo usado para a sua transposição. Elementos da Classe I são TEs que se movimentam através de um intermediário de RNA que é transcrito, reversamente, por uma transcriptase reversa antes da nova inserção. Estas

seqüências são chamadas de retrotransposons e incluem os retrotransposons com LTRs (longas repetições terminais), dos quais alguns são estruturalmente similares aos retrovírus, e elementos sem LTRs ou retroposons. Elementos da Classe II movem-se via um intermediário de DNA através de um mecanismo de excisão do sítio original e reinserção do elemento em outro ponto do genoma. Mais recentemente, um grupo peculiar de elementos de Classe II foi descoberto e denominado MITES (Miniature Inverted-repeat TEs) os quais se caracterizam por sua defectividade, tamanho normalmente pequeno e alto número de cópias no genoma (revisão em Feschotte *et al.*, 2002). Elementos da Classe II têm sido encontrados tanto em procariotos como em eucariotos, enquanto retrotransposons e MITES parecem estar restritos aos eucariotos (Capy *et al.*, 1998, Feschotte *et al.*, 2002).

Ainda, em relação à sua presença, famílias e subfamílias diferentes de TEs podem coexistir num mesmo genoma (Flavell, 1992; Clark *et al.*, 1994; Lohe *et al.*, 1995), e o número de famílias e de cópias de cada família varia conforme a espécie hospedeira. A capacidade de mobilização e, portanto, as implicações biológicas dos TEs são geralmente distintas dependendo se sua localização cromossômica for eucromática ou heterocromática. Cópias ativas são encontradas principalmente na eucromatina, enquanto que na heterocromatina observamos um maior número de TEs, sendo muitas cópias defectivas e rearranjadas. No entanto, permanece ainda a dúvida se existe

uma preferência insercional de TEs por determinadas regiões ou se este padrão é resultado de diferentes pressões seletivas (Capy *et al.*, 1998).

A origem de uma família de TEs dentro do genoma de uma espécie pode ocorrer de três maneiras: por eventos de mutação e recombinação de seqüências já presentes no genoma (Finnegan, 1989); por meio de transmissão horizontal (Daniels *et al.*, 1990b); ou ainda por meio de hibridização introgressiva e poliespermia entre espécies aparentadas (Capy *et al.*, 1998). Posteriormente, os novos integrantes do genoma podem se espalhar por transmissão vertical às populações a partir de linhagens ascendentes. Uma vez estabelecidas no genoma, ocorre uma rápida propagação e aumento do número de cópias dessas seqüências. Nesse momento, as interações que se estabelecem entre o TE e o genoma são muito importantes para a sobrevivência de ambos. A taxa, na qual este fenômeno ocorre, depende de vários fatores que regulam a transposição do elemento, como a sua capacidade de invasão, as características do genoma hospedeiro, fatores ambientais e interação entre todos estes fatores (Pinsker *et al.*, 2001). A fase seguinte do ciclo de vida de um TE é a de inativação e silenciamento que pode ser caracterizada pelos eventos de redução crescente da mobilidade do elemento em razão do acúmulo de cópias defectivas, e pelo estabelecimento de mecanismos epigenéticos de silenciamento desenvolvidos pelo hospedeiro (Pinsker *et al.*, 2001). A partir daí, a família do TE pode seguir algum dos possíveis destinos como: período de senescência,

caracterizada por eventos de degradação dessas seqüências pelo acúmulo de mutações, seguida da provável perda estocástica do elemento; ressurreição do TE, previamente silenciado, por eventos de recombinação ectópica entre elementos; recrutamento do elemento pelo genoma hospedeiro para o desenvolvimento de alguma função que beneficie a espécie; e ou novo evento de transmissão horizontal para um genoma ainda desprotegido. Um modelo mais específico dentro desse panorama geral sobre a dinâmica de TEs é exemplificado através do ciclo de vida de um transposon (Brookfield, 2005), na Figura 1.

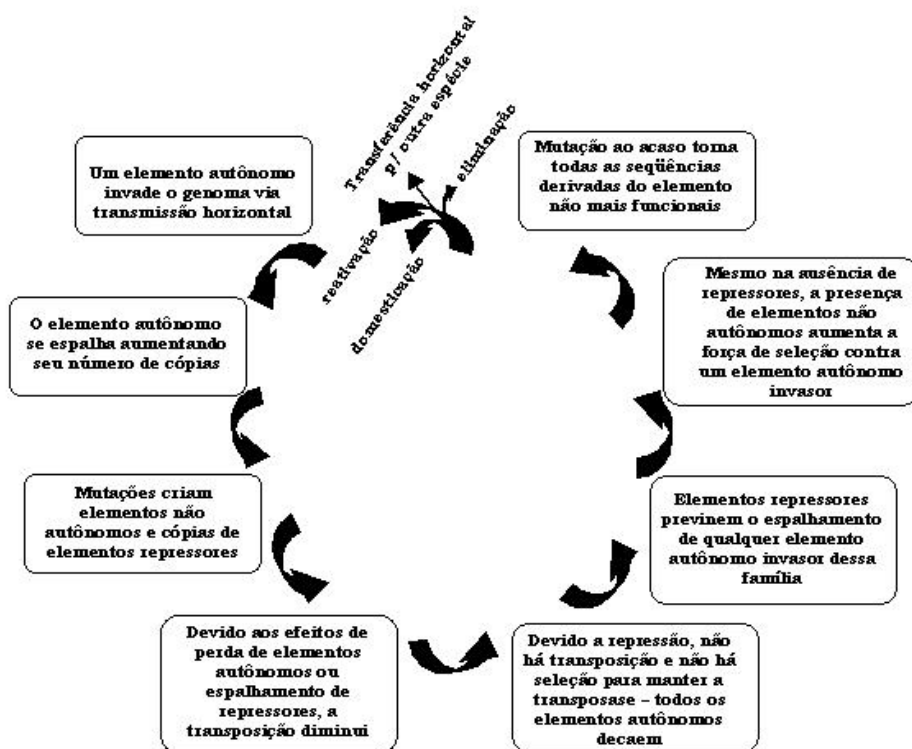


Figura 1 - Ciclo de vida de um transposon. Primeira fase: invasão por transferência horizontal. Segunda fase: propagação e aumento do número de cópias. Terceira fase: inativação e silenciamento e possíveis destinos para essas seqüências. Adaptado de Brookfield (2005) e Almeida e Carareto (2005).

Por outro lado, os TEs podem ter um impacto positivo no genoma hospedeiro promovendo um aumento da variabilidade genética. Este aumento na variabilidade genética é devido à sua movimentação (transposição) ou à recombinação homóloga entre cópias de elementos de uma mesma família. Como resultado destes fatores, temos mudanças que vão desde mutações pontuais até modificações no tamanho e no arranjo de genomas inteiros. Assim, estas mudanças dão-se tanto na estrutura (rearranjos cromossômicos) como na função (organização e mudança na expressão de genes) do genoma. Através dessas mudanças no genoma, os TEs parecem ter alcançado, ao longo de milhões de anos de evolução, um balanço entre os efeitos deletérios nos indivíduos e um efeito benéfico a longo prazo nas espécies. Se essas seqüências repetidas são hoje DNA lixo, permanece uma questão complexa. Algumas podem ter tido uma função importante há muito tempo, mas perderam essa função hoje e outras podem nunca ter tido uma função. Mesmo assim, o acúmulo dessas seqüências “desordenando” nossos genomas é ainda um pequeno preço pago se levarmos em conta a plasticidade que elas propiciam.

A inserção de TEs pode ativar ou inativar genes dependendo do local de sua inserção (dentro de genes ou em suas regiões regulatórias). Eles também podem promover inversões e deleções de DNA cromossômico, ou como um resultado direto da transposição intramolecular, ou por promover regiões dispersas de homologia que podem ser reconhecidas pela maquinaria da

recombinação. A transposição de alguns elementos pode resultar na transdução de DNA flanqueador e, assim, promover ainda outros meios de rearranjar os genes do hospedeiro. Finalmente, os TEs podem contribuir ainda, dependendo das suas seqüências, com funções adicionais aos genomas como, por exemplo, resistência a antibióticos e fatores de virulência em bactérias. Em eucariotos, funções associadas aos TEs têm sido co-optadas pelo hospedeiro para alterar a estrutura do genoma e a expressão gênica, conforme evidenciado pela recombinação V(D)J, *splicing* de introns nucleares e manutenção de telômeros. (Agrawal *et al.*, 1998; Hiom *et al.*, 1998; Sharp, 1991; Eickbush, 1997).

Sendo assim, os elementos transponíveis hoje são aceitos como um importante fator evolutivo e como seqüências capazes de reorganizar o genoma das espécies que os carregam. Por isto, estas entidades são evocadas em discussões que abordam os mais diferentes temas de interesse sobre a organização biológica. Pesquisadores tentam entender a dimensão do papel dos elementos móveis e suas respectivas funções para a manutenção da vida, investigando sua distribuição e representatividade nos mais diferentes genomas, as relações evolutivas entre eles e deles com o genoma hospedeiro, além de sua função como vetor de troca de informação genética entre os seres vivos.

O elemento transponível *hobo*

O elemento transponível *hobo* foi originalmente descrito como uma mutação de inserção no gene *Sgs-4* de *Drosophila melanogaster* (McGinnis *et al.*, 1983). Estruturalmente, os TEs da família *hobo* são classificados como elementos de Classe II de TEs de eucariotos, transpondo-se via um intermediário de DNA (Finnegan, 1989). Pertencem à superfamília *hAT* de TEs, denominada de acordo com seus 3 principais membros: *hobo* de *D. melanogaster*, *Ac* de *Zea mays* e *Tam3* de *Anthriscum majus*. Todos os elementos *hAT* compartilham algumas características definidas, incluindo: curtas repetições terminais invertidas (ITRs) em cada uma das extremidades do elemento, a geração de uma duplicação de 8 pares de bases no sítio alvo da transposição, e possuem um gene que codifica uma transposase que cataliza os passos de clivagem do DNA e junção do transposon no novo sítio. Esta superfamília inclui também um número de elementos relacionados encontrados em outros gêneros de insetos (Calvi *et al.*, 1991; Atkinson *et al.*, 1993; Warren *et al.*, 1994), vermes (Bigot *et al.*, 1996) e fungos (Okuda *et al.*, 1998), sendo também bastante abundante em plantas (Kunze e Well, 2002).

O elemento *hobo* canônico (HFLI), isolado de *D. melanogaster* tem um tamanho de 2959 pb, curtas ITRs de 12 pb, uma ORF de 1,9 kb que codifica sua transposase e, assim como os demais elementos de sua superfamília, duplicam 8 pb no sítio de sua inserção. Apresentam, ainda, alguma

variabilidade no número de cópias de uma repetição de 9 pb (repetição S ou TPE) no centro da região codificante, à qual está possivelmente associada a habilidade invasiva do elemento. (Calvi *et al.*, 1991; Bazin e Higuët, 1996; Souames *et al.*, 2003). Dentre os diferentes sítios de restrição que esse elemento possui, dois sítios de restrição de *Xho*I próximos a cada extremidade são muito importantes para as análises, através de Southern blot, de sua integridade no genoma.

Dos TEs conhecidos de *Drosophila*, *hobo* é o que apresenta uma das distribuições mais restritas e o padrão de distribuição de seqüências relacionadas a este elemento, nesse gênero, pode nos dar algumas pistas importantes sobre seu passado recente. Daniels *et al.*, (1990^a) detectaram seqüências homólogas a *hobo* somente nos subgrupos *melanogaster* e *montium*, ambos pertencentes ao grupo *melanogaster* do subgênero *Sophophora*. Os autores mostraram, ainda, que elementos *hobo* completos estão restritos aos membros do complexo *melanogaster*, um grupo de espécies crípticas que inclui *D. melanogaster*, *D. simulans*, *D. mauritiana* e *D. sechellia* (Daniels *et al.*, 1990^a; Periquet *et al.*, 1990) e, desses, somente um subconjunto é esperado ser ativo. Entretanto, Loreto *et al.* (1998^a) mostraram uma fraca hibridização com sonda de *hobo* em algumas linhagens de *D. willistoni*, a qual pertence a outro grupo do mesmo subgênero, levando-os a sugerir que esse TE pode ter uma distribuição menos restrita. Fora do gênero *Drosophila*, elementos do tipo *hobo* (*hobo*-like) foram encontrados em

diferentes espécies de tefritídeos (Handler e Gomez, 1996; Torti *et al.*, 2005) e em algumas espécies de lepidópteros (DeVault e Narang, 1994; Borsatti *et al.*, 2003).

O elemento *hobo* no complexo *melanogaster*

Como para a maioria dos TEs e em função da descoberta de *hobo*, a espécie mais estudada e, provavelmente, a melhor caracterizada para esse elemento é *Drosophila melanogaster*. Muitos estudos populacionais investigando a presença/ausência desse elemento no genoma, estimativas de número de cópias, integridade, funcionalidade, capacidade invasiva, conservação de seqüência, entre outros, foram inicialmente desenvolvidos nessa espécie e, portanto, tem-se nela um modelo de “comportamento” de *hobo* no genoma de *Drosophila*.

Algumas linhagens de *D. melanogaster*, quando avaliadas por Southern blot, contêm em seus genomas elementos *hobo* completos (~3kb) e menores (internamente deletados), enquanto outras não (Streck *et al.*, 1986). As linhagens que possuem cópias completas e deletadas são descritas como H (de “*hobo*-containing”) e as que não possuem essas cópias são descritas como E (de “empty”). Desde as análises populacionais iniciais de *hobo* nessa espécie uma característica marcante tem sido observada: além das cópias completas e internamente deletadas, os genomas de todas as linhagens

possuem seqüências que hibridizam com sonda de *hobo* em estringências baixa a moderada, porém, numa estringência maior estas bandas se tornam menos intensas. Estas cópias são denominadas seqüências relacionadas a *hobo* e sugere-se que as mesmas possam ser remanescentes de introduções mais antigas de *hobo* no genoma (Pascual e Periquet, 1991) o que leva a serem descritas, também, como *hobo* relíquias, ou simplesmente “relics”. Elas podem ser distingüidas das seqüências canônicas por 2 características nas análises por Southern blot. Primeiro, a digestão de elementos *hobo* canônicos com a enzima de restrição *Xho*I produz bandas intensas correspondendo ao acúmulo dos fragmentos internos de elementos com a mesma estrutura. Elementos completos de 3 kb produzem uma banda de 2,6 kb, e cada classe particular de elementos defectivos produz uma banda característica de tamanho menor. Em contraste, seqüências relacionadas a *hobo* (“relics”) produzem numerosas bandas fracas de alto peso molecular, que variam de linhagem para linhagem, provavelmente por terem perdido um ou ambos os sítios de restrição de *Xho*I. Segundo, seqüências relíquias normalmente necessitam de tempos de exposição maiores da membrana ao filme de raio-X para serem melhor detectadas. Ainda, seqüências *hobo* “relics” diferem claramente dos elementos *hobo* internamente deletados, que são comuns em linhagens H de *D. melanogaster*, como os elementos *Th1* e *Th2* (1,51 e 1,49 kb respectivamente). Cópias internamente deletadas típicas diferem do elemento completo por apresentarem uma única grande deleção,

que remove uma parte central da seqüência, mas não afeta as extremidades do elemento (revisão em Periquet *et al.*, 1994), enquanto que seqüências *hobo* “relics” normalmente apresentam muitos “indels” e sofrem rearranjos mais complexos.

Em relação ao padrão temporal e geográfico de distribuição de elementos *hobo* no genoma de *D. melanogaster*, estudos de linhagens mais antigas, coletadas antes de 1950, mostram que elementos canônicos estavam ausentes nestas linhagens. Já as linhagens recentemente coletadas possuem o elemento, sugerindo que este foi recentemente introduzidos nesta espécie por transferência horizontal. A maioria das linhagens antigas (coletadas antes de 1950) foram E, enquanto linhagens recentes de todas as partes do mundo foram H (Periquet *et al.*, 1989^a, 1989^b, 1990; Boussy e Daniels, 1991; Pascual e Periquet, 1991).

Em *D. simulans*, o elemento *hobo* também é encontrado sob suas três formas estruturais (Streck *et al.*, 1986; Boussy e Daniels, 1991). Simmons (1992) demonstrou que o elemento completo de *D. simulans* é virtualmente idêntico ao elemento canônico de *D. melanogaster* em sua seqüência de DNA, diferindo somente em três posições em 2563 sequenciadas (o fragmento interno de *Xho*I). Elementos completos nesta espécie são de aproximadamente 3 kb de tamanho e elementos internamente deletados típicos também estão presentes, sendo *hdelsim* (1,08 kb) o mais freqüente deles (Boussy e Daniels, 1991). Além de carregar cópias completas e

internamente deletadas, *D. simulans* também carrega muitas cópias de seqüências relacionadas a *hobo*. Estas seqüências mostram as mesmas características daquelas descritas para *D. melanogaster* nas análises por Southern blot, embora tenham sido até o momento menos bem caracterizadas em *D. simulans* do que foram em *D. melanogaster*.

O padrão de distribuição temporal e geográfico de *hobo* em *D. simulans* também mostra que todas as linhagens coletadas recentemente contêm elementos completos (linhagens H). Entretanto, o padrão para linhagens coletadas antes de 1950 não é tão claro como para *D. melanogaster*. Boussy e Daniels (1991) analisaram 27 linhagens de *D. simulans* e encontraram somente quatro desprovidas de elementos completos (linhagens E), uma de origem desconhecida e três linhagens antigas coletadas na América do Sul. Isto pode ser interpretado como uma invasão mais recente de elementos *hobo* nas populações da América do Sul, mas a amostragem geral foi muito limitada para mostrar algum padrão geográfico de distribuição, e a presença de linhagens E em *D. simulans* permanece como uma questão aberta. No entanto, Loreto *et al.* (1998c) aumentaram o número de populações sul americanas analisadas e encontraram poucas linhagens antigas de laboratório com sinal pouco intenso correspondente ao elemento completo. Além disso, curiosamente, todas as populações analisadas foram desprovidas da cópia internamente deletada típica de 1,1 kb, sempre presente nas linhagens de outras partes do mundo (Boussy e Daniels, 1991). Estes

achados reforçam a hipótese de introdução recente desse elemento nas populações sul americanas. Embora os estudos mencionados anteriormente corroborem o padrão temporal e concordem com a possibilidade de uma introdução recente e espalhamento de seqüências *hobo* em *D. melanogaster*, eles levantam dúvidas sobre a direção da transferência (proposto anteriormente como *D. simulans* sendo a provável espécie doadora) e, de fato, sobre a validade de qualquer explicação simples sobre a direção da transferência e o tempo de invasão desse elemento.

Em *D. mauritiana* e *D. sechellia* as análises por Southern blot mostram padrões semelhantes àqueles observados para linhagens H de *D. melanogaster* e *D. simulans*, indicando que elementos completos, internamente deletados e seqüências relacionadas a *hobo* estão presentes nos seus genomas (Daniels *et al.*, 1990a; Periquet *et al.*, 1990; 1994). Simmons (1992) também seqüenciou um elemento completo de *D. mauritiana* e demonstrou que ele é quase indistinguível em relação à seqüência dos elementos completos de *D. melanogaster* e *D. simulans*. Embora o fragmento de 2,6 kb de *XhoI* esteja presente nas análises por Southern blot em *D. sechellia*, Periquet *et al.* (1994) não conseguiram detectar elementos completos por PCR, mas amplificaram e seqüenciaram um *hobo* pequeno (1,1 kb). Os 309 pb da região 5' deste elemento foram 95% idênticos a HFLI, e os 435 pb da região 3' foram 97% idênticos a HFLI, mas a porção entre eles não apresentou nenhum padrão óbvio de similaridade a *hobo*. Este elemento foi

denominado *hdelsech* e seu baixo grau de identidade com *hobo* canônico sugere que ele seja uma seqüência relacionada a *hobo*, comum às demais espécies do complexo (Boussy e Itoh, 2004).

O elemento *hobo* fora do complexo *melanogaster*

A presença, abundância e as diferentes formas de integridade do elemento *hobo* observadas nas espécies do complexo *melanogaster* não são assim tão óbvias para as outras espécies do subgrupo e do grupo *melanogaster*. Além disso, pouco se sabe e se tem investido em saber a respeito dessas seqüências nessas outras espécies, daí a escassez de informações sobre a dinâmica de *hobo* fora do complexo *melanogaster*. Dados disponíveis são em relação a análises por Southern blot do DNA genômico hibridizado com sonda de *hobo* canônico.

D. yakuba, *D. teissieri*, *D. erecta* e *D. orena*, as quais também fazem parte do subgrupo *melanogaster* mas não do complexo *melanogaster*, contêm uma ou poucas seqüências que hibridizam com sonda de *hobo* mas não mostram os fragmentos de restrição esperados do elemento *hobo* completo (Daniels *et al.*, 1990a; Periquet *et al.*, 1994).

No subgrupo *montium*, o qual foi descrito como carregar também elementos *hobo*, 21 das 26 espécies testadas mostraram uma ou mais bandas de hibridização, mas nenhuma mostrou os tamanhos de fragmentos

de restrição que corresponderiam ao elemento canônico (Daniels *et al.*, 1990a).

Seqüências relacionadas a *hobo*

A respeito da sua presença e aparência peculiares e, pelo fato de que elas podem nos fornecer informações valiosas sobre a origem, história e dinâmica de manutenção do elemento *hobo*, recentemente têm sido feitos mais esforços na tentativa de caracterizar as seqüências relacionadas a *hobo* (ou relíquias). Simmons *et al.* (1998) e Galindo *et al.* (2001) descreveram seqüências relíquias de *D. melanogaster* como não funcionais e incapazes de codificar uma transposase. Estas seqüências mostraram vários rearranjos em comparação com HFLI e, além disso, apresentaram numerosas inserções/deleções (“indels”) e substituições. O grau médio de identidade de seqüência destes elementos em relação a HFLI em regiões homólogas foi de aproximadamente 85% (variando de 80-92%). Esses autores sugerem que essas seqüências relíquias são imóveis e distantemente relacionadas ao *hobo* moderno. Boussy e Itoh (2004) isolaram e seqüenciaram um elemento relíquia do genoma de *D. melanogaster* e mostraram que, além de não ser capaz de codificar uma transposase, esse elemento provavelmente seria incapaz de se transpor na presença de uma transposase funcional, tal é seu grau de degeneração. Sua ITR de 12 pb da região 3’ está intacta mas, em contraste, a ITR da região 5’ está extremamente degenerada. Até

recentemente, quase todas as seqüências relacionadas a *hobo* descritas eram degeneradas, não-funcionais e consideradas como elementos provavelmente imóveis e imobilizáveis (ver artigo1). Além disso, das poucas seqüências caracterizadas, a maioria foi isolada do genoma de *D. melanogaster* e pouca atenção tem sido dada ao genoma de *D. simulans*. No entanto, independente da espécie, se estas seqüências habitam os genomas de diferentes linhagens por tanto tempo e ainda mantêm estrutura suficientemente conservada a ponto de serem detectadas e isoladas, podemos levantar algumas questões interessantes sobre o comportamento das mesmas. Por exemplo, são mesmo seqüências imobilizáveis ou seriam capazes de sofrer mobilização *in trans*? Se isso for possível, qual a fonte de transposase e quais os requisitos estruturais necessários para tal mobilização? Mais curiosamente, ainda, como estas seqüências têm se mantido por tanto tempo nos genomas, qual sua verdadeira origem e destino e, em última instância, qual seu papel na evolução do genoma no qual se hospeda? Esta tese, de maneira geral é uma tentativa de contribuição às respostas a algumas dessas perguntas, embora muitas questões permaneçam, ainda, em aberto, até que novos estudos possam ajudá-las a serem respondidas.

OBJETIVOS

O objetivo do presente trabalho foi estudar o comportamento de seqüências relacionadas a *hobo* no genoma de *D. simulans*, na tentativa de contribuir para o entendimento da dinâmica dessas seqüências nos genomas que elas habitam. Para isto, alguns objetivos específicos foram propostos, como:

CAPÍTULO 2

- isolar e caracterizar uma seqüência relacionada a *hobo* envolvida no surgimento de uma mutação *white de novo* em uma linhagem hipermutável de *D. simulans*;

CAPÍTULO 3

- analisar a presença, número de cópias, integridade, estabilidade e similaridade da seqüência do elemento *hobo* no genoma de diferentes populações sul americanas de *D. simulans* em comparação ao elemento canônico;

CAPÍTULO 2

Mobilization of a *hobo*-related sequence in the genome of *Drosophila simulans*

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Mobilization of a *hobo*-related sequence in the genome of *Drosophila simulans*

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Key words: canonical *hobo*, cross mobilization, *hobo* relic, hypermutable strain, transposable elements

Abstract

The *hobo* transposable element can occur under three forms in the *Drosophila* genome: as a complete element (also called canonical), as internally deleted copies, or as *hobo*-related sequences (relics). Some evidence indicated that canonical elements and internally deleted copies are recent acquisitions of *Drosophila* genomes, while the “relics” are old components, normally degenerated and immobile. Here we present the characterization of a *hobo*-related sequence, found in the genome of a hypermutable strain of *D. simulans*, which insertion into the *white* locus raised a *de novo white* mutation. It is a shorter *hobo* related element presenting, overall, roughly 18% of divergence at the DNA level from the canonical *hobo*, with many indels that make clear this element is defective. However, its ITRs and flanking regions are extremely conserved. This is the first *hobo* “relic” showed to be mobilizable. We suggest, and point up some evidences, toward the idea that this sequence could have been mobilized by the canonical element. The presence of a similar “relic” element in *D. sechellia* allows us to suggest that these elements have been maintained mobilizable since the time of divergence between these species.

Introduction

hobo is a Class II DNA transposon (Finnegan, 1989) with short inverted terminal repeats (ITRs) (McGinnis, Shermoen and Beckendorf, 1983; Streck, MacGaffey and Beckendorf, 1986). The canonical *hobo* (HFL1), isolated from *D. melanogaster*, has ITRs of 12 bp and encodes a transposase (Calvi et al., 1991). Insertion sites of *hobo* elements are typically flanked by an 8 bp duplication of host sequence. The *hobo* element has one of the narrowest distributions of any *Drosophila* transposon so far examined. Daniels, Chovnick and Boussy (1990) assayed 134 species by Southern blot and demonstrated that within

the genus *Drosophila* only the *melanogaster* and *montium* species subgroups contain *hobo* elements sufficiently conserved to allow detection. Moreover, only the *melanogaster* sibling species (*D. melanogaster*, *D. simulans*, *D. mauritiana*) contained large numbers of elements, and only a subset of those are expected to be active. However, Loreto et al. (1998a) showed a weak hybridization with the *hobo* probe in some strains of *D. willistoni*, which belong to the other group, suggesting that this transposable element (TE) could have a not so narrow distribution. Out of *Drosophila* genus, *hobo*-like elements have been found in some species of Diptera, like *Musca domestica* (Atkinson, Warren and O’Brochta, 1993) and different

tephritids (Handler and Gomez, 1996; Torti et al., 2005) and, also in some species of Lepidoptera (DeVault and Narang, 1994; Borsati, Azzoni and Mandrioli, 2003). In animals other than insects, *hobo*-like elements have been identified only in the nematode *Caenorhabditis elegans*.

The canonical *hobo* elements have two *Xho*I restriction sites close to each end, which are very important for Southern blot analysis. In *D. melanogaster*, the genomes of some strains contain full-size and smaller (internally deleted) *hobo* elements, but others have none (Streck, MacGaffey and Beckendorf, 1986). The former are described as “H” (for “*hobo*-containing”) and the latter as “E” (for “empty”). In the earliest *D. melanogaster* population analysis of *hobo* by Southern blot, a curious feature was described. In addition to full-size and smaller (internally deleted) *hobo* elements, all genomes contained sequences that hybridized with a *hobo* probe at low to moderate stringencies. At higher stringency, these bands faded, and, based on the sizes of the bands, the sequences clearly lacked one or both of the *Xho*I sites of the canonical sequence (Boussy & Daniels, 1991). These sequences were called *hobo*-

related sequences or “relics”. Simmons et al. (1998) demonstrated that these sequences usually diverge 10–20% from the canonical *hobo* at DNA level. Figure 1 shows a diagrammatic representation of three forms of *hobo* elements so far described from the *Drosophila* genomes.

In relation to the temporal and geographical patterns of distribution of *hobo* elements, surveys of older *D. melanogaster* strains, collected before 1950, pointed out the complete *hobo* elements were absent in those strains (E), whereas all recent strains from all parts of the world were H. These findings suggest this element have recently been introduced into this species by horizontal transfer (Periquet et al., 1989a, b; 1990; Boussy & Daniels, 1991; Pascual & Periquet, 1991).

The *hobo* element is also found in *D. simulans* under its three forms (Streck, MacGaffey & Beckendorf, 1986; Boussy & Daniels, 1991). Simmons (1992) showed that most of a full-size *hobo* from *D. simulans* was virtually identical in DNA sequence to the canonical *hobo* of *D. melanogaster*, differing at only three positions out of 2563 sequenced (the internal *Xho*I fragment). Complete elements in this species are approximately 3 kb in

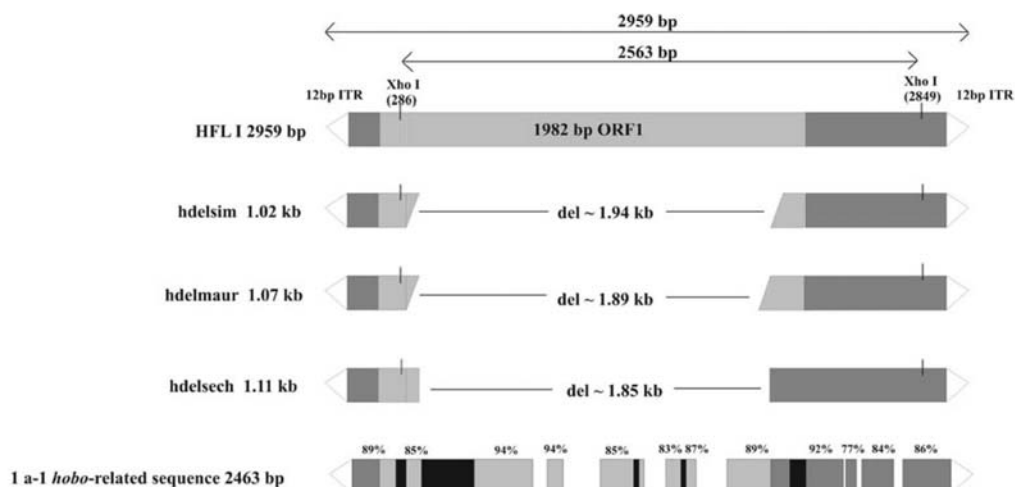


Figure 1. Three possible forms of *hobo* elements. Structure of the full-size *hobo* element HFL1, some common internally deleted elements in *D. simulans* (*hdelsim*), *D. mauritiana* (*hdelmaur*) and *D. sechellia* (*hdelsech*), and a *hobo*-related sequence in *D. melanogaster* (1a-1). ORF 0 is just to the left of ORF 1 (both light gray). The difference between ORF0 and the untranslated regions is not clear. At 1a-1 *hobo*-related sequence: degree of identity with the HFL1 is indicated above the relevant portions; insertions are indicated as darkened regions. Restriction site is indicated for *Xho*I. The 2.6 kb fragment generated from a full-size element by *Xho*I digestion is indicated. Adapted from Periquet et al. (1994) and Boussy and Itoh (2004).

length and typical deleted elements are also present, and *h del sim* (1080 bp, Figure 1) being the most frequent one (Boussy & Daniels, 1991). Besides carrying full-size and smaller *hobo* elements, *D. simulans* also carries many copies of *hobo*-related sequences detected on Southern blot. These last sequences show the same characteristics as those described to *D. melanogaster* in a Southern blot analysis, although none of these copies have been sequenced until now.

The temporal and geographical distribution patterns of *hobo* in *D. simulans* hold similarities with those described for *D. melanogaster*, since all recently collected strains contain complete *hobo* element (H strains). However, the pattern for strains collected before 1950 is not so clear as for *D. melanogaster*. Boussy and Daniels (1991) performed an analysis of 27 strains and found only 4 empty for complete *hobo* (E). For this reason the authors suggested that *D. simulans* could be the donor of *hobo* to *D. melanogaster*, since *hobo* was apparently present in the *D. simulans* genome before it was in *D. melanogaster*.

D. mauritiana and *D. sechellia* in a Southern blot analysis display patterns similar to those observed for *D. melanogaster* and *D. simulans* H strains, implying that full-size, internally deleted and *hobo*-related sequences are present (Daniels, Chovnick and Boussy, 1990; Periquet et al., 1990, 1994). Simmons (1992) also sequenced a full-size *hobo* from *D. mauritiana* and showed it is almost indistinguishable in sequence to the canonical *hobo* of *D. melanogaster* and to the full-size *hobo* of *D. simulans*. Periquet et al. (1994) were unable to detect a full-size *hobo* by PCR in *D. sechellia*, despite the presence of a 2.6 kb *XhoI* fragment on Southern blots, but they amplified and sequenced a small (1107 bp) *hobo*. The 5' 309 bp of this element were 95% identical to HFL1, and the 3' 435 bp were 97% identical, but the portion between them bore no obvious similarity to *hobo*. This element was named *hdelsech* (Figure 1) and its lower degree of identity with canonical *hobo* suggests that it is a *hobo*-related element.

More recently some effort has been done to characterize the *hobo* "relics". The main reason for it is that these sequences may provide us with valuable information about the origin of *hobo*, its history and maintenance dynamics. Simmons et al. (1998) and Galindo et al. (2001)

have described *D. melanogaster hobo*-related sequences as non-functional and unable to codify a transposase. These sequences show various rearrangements in comparison to HFL1 and, moreover, present numerous indels and substitutions. The average degree of sequence identity of these elements to HFL1 in homologous regions was approximately 85% (range: 80–92%). These authors suggested these *hobo* "relics" are ubiquitous, immobile and distantly related to modern *hobo*. Boussy and Itoh (2004) have recently sequenced a *hobo*-related sequence from the genome of *D. melanogaster*. This described sequence is clearly unable to encode a functional transposase, and is probably incapable to transpose in the presence of a functional transposase. Its 3' 12 bp ITR is intact but the 5' ITR is, in contrast, extremely degenerate. Until now, all *hobo* related sequences described are degenerated, non-functional and probably immobile elements. However, some questions subsist unanswered. For example: are there "relic" *hobos* able to be mobilized in *trans*? If so, what is the transposase source and the structural features required for mobilization? How are the "relics" maintained in the genomes?

The detection and monitoring of a *de novo* mutation in some strains give the opportunity to investigate the nature of the causal agent of the mutations. When this agent appears to be a transposable element, we can also investigate the nature of its mobilization. Loreto et al. (1998b) detected and accompanied a hypermutable strain of *D. simulans* established from a male spontaneous mutant, captured in nature, along roughly 100 generations of observation. From this original strain, some mutations, both autosomal and X-linked, were isolated. Among the mutant sub-strains established from the former hypermutable strain, we chose a *white* mutant for molecular characterization. The purpose of the present study was to investigate the nature of the underlying agent of the *de novo white* mutation. We show here that a *hobo*-related sequence insert into the *white* locus of this *D. simulans* mutant caused this mutation. In particular, we wanted to determine the molecular structure of the *hobo*-related sequence that was mobilized in the genome of this strain of *D. simulans* suggesting then the possibility of cross mobilization with the canonical *hobo* element in this species.

Materials and methods

Fly stocks

The *Drosophila simulans white* mutant (Dswhm) used in this work was originated from a hypermutable strain of *D. simulans* described by Loreto et al. (1998b). This mutation appeared in the 81st generation of this hypermutable strain. So, it is a well-characterized *de novo* mutation. For Southern blot analysis, two other strains were used as control, a wild-type strain (*Eldorado*), and another mutant for *lozenge* locus, but with normal eye color (wild-type to the *white* gene).

Southern blot analysis

Genomic DNA was prepared from approximately 100 adult flies according to Jowett (1986). DNA samples were digested with restriction enzymes following manufacturer directions (Invitrogen). The enzymes employed were *HindIII*, *SalI*, *BamHI*, *EcoRI*, *BglII* and *SacI* according to the restriction map of the *white* locus of *D. simulans* (Inoue and Yamamoto, 1987; Inoue, Taira and Yamamoto, 1988).

DNA fragments were separated by electrophoresis on 1% agarose gels and transferred to nylon membranes (Hybond N+ /Amersham Biosciences). The membranes were hybridized to a random primer-labeled probe at 60°C in 5× SSC; 0.1% SDS; 5% dextran sulfate; and 20-fold dilution of liquid block. The filters were washed twice with 0.2× SSC and 0.5% SDS for 15 min at 60°C. Hybridization and detection were performed using a Gene Images Kit (Amersham Biosciences) according to manufacturer instructions. The *pCaSpeR-hs* plasmid (GenBank U59056) which has a mini-*white* gene of *D. melanogaster* (Thummel and Pirrota, 1992) was used as a probe for hybridization.

DNA amplification and sequencing

PCR reactions were performed in 25 µl volumes using approximately 20 ng of template DNA, 20 pmol of each primer, 1.5 mM of MgCl₂, 50 µM of each nucleotide and 1 U of *Taq* DNA Polymerase (Invitrogen) in 1× Polymerase buffer. After an initial denaturation for 2 min at 95°C, 35 cycles consisting of 1 min denaturation at 95°C, 40 s annealing at 55°C and 2 min extension at 72°C were

carried out. An additional extension step of 5 min at 72°C was performed after the last cycle. The primers used (WT1: 5'-CGGTGAGTTTCTATT CGCAA-3' and WT2: 5'-GTCCCACCAACTA-CAATCCG-3') anneal into the *white* locus sequence of *D. simulans* flanking the third exon. The amplified fragment of the *white* mutant strain containing the insert was separated by electrophoresis on 1% agarose gel. The PCR product was purified and cloned into pCR-TOPO[®] plasmid (Invitrogen). DNA sequencing was performed directly from the purified plasmids in a MegaBACE 500 automatic sequencer. The dideoxy chain-termination reaction was implemented with the use of the DYEnamic ET[®] kit (Amersham), the WT1 and WT2 primers plus two internal primers. The sequences were then submitted to a "confidence consensus" analysis with the use of the Staden Package Gap 4 program (Staden, 1996) and a consensus sequence was obtained. BLAST analyses were performed using search engines at the Berkeley *Drosophila* Genome Project (BDGP; www.fruitfly.com) and GenBank (www.ncbi.nlm.nih.gov) to look for sequences with general similarity. Then, the resultant sequences were aligned using ClustalX (Thompson et al., 1997). The sequences accession numbers are: *D. melanogaster* (HFL1)= M69216; *D. sechellia* (*hdelsech*)= X77577 and *D. mauritiana* *mkg* AY562976 and AY562984. The *D. simulans hobo*-related sequence described in this work was deposited under number AY764286.

Results

Genomic characterization of the *white* mutation

The *D. simulans* genomic region of the *white* gene has been depicted by Inoue and Yamamoto (1987) and Inoue, Taira and Yamamoto (1988). Using these restriction maps as a model, the genomic region of *D. simulans white* mutant (Dswhm) was characterized. As can be seen in Figure 2, the restriction sites in the *white* strain (Dswhm) are similar to the described restriction maps. However, an insertion of 1.2 kb was detected, into the third exon, between the *BglII* and *BamHI* sites. Although six different restriction enzymes were used to assemble this map, only blots of two of them (*BglII* and *SacI*) were presented in the Figure 3, since they were the more representative of

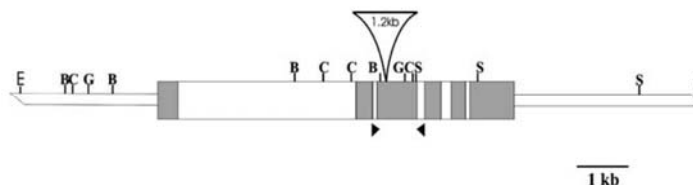


Figure 2. Partial restriction map of the *white* locus of *D. simulans* *white* mutant. Gray boxes from left to right denote the exons 1–6. Unshaded boxes denote the introns 1–5. The insert of *hobo*^{v-a} within the exon 3 is represented by the triangle above. Primers used to isolate the insert are represented by the triangles below the restriction map. Restriction enzyme abbreviations: E, *Eco*RI; B, *Bam*HI; S, *Sal*I, C, *Sac*I, G, *Bgl*II.

the differences observed. As can be seen in Figure 3(a), when the genomic DNA from both wild type strains or from *white* mutant was digested with *Bgl*II, all fragments observed correspond to the expected size. An exception was the 5.3 kb fragment that showed an enlargement to 6.5 kb in the *white* mutant, meaning an insertion of 1.2 kb into this region. When the genomic DNA was digested with *Sac*I, all the fragments also correspond to expected sizes, with the exception of one fragment in the *white* mutant, that shows a 2.4 kb band instead of the expected 1.2 kb (Figure 3(b)). Therefore, it was possible to localize a 1.2 kb

insertion, in the *white* mutant, precisely between *Bgl*II and *Bam*HI sites.

Sequence analysis of insertion

A fragment with roughly 1800 bp was amplified by PCR employing primers annealing close to the insertion present in the *Dswm* mutant. The sequencing of this fragment showed beyond *white* gene region, a *hobo*-related sequence of 1220 bp long that does not contain any functional ORFs; hence, been unable to produce a transposase and to carry out autonomous mobilization. However, its 12 bp inverted terminal repeats (ITRs) were well conserved when compared with those of canonical *hobo* element (HFL1). The 5' ITR was intact and the 3' ITR shows just one nucleotide substitution. Furthermore, flanking the *hobo*-related sequence there was an 8bp duplication of the *white* gene sequence. This duplication is typical from *hobo* insertions. We call this *hobo*-related sequence as *hobo* “velho-assanhado” (*hobo*^{v-a}), which means in Portuguese “an elder very animated”. As will be further discussed, this sequence has apparently been present in the *D. simulans* genome for a long time (therefore elder) but is still mobilizable and could be related to the hypermutability phenomenon (animated).

The comparison among *hobo*^{v-a} with other *Drosophila hobo* sequences described hitherto shows variable levels of similarity. Some comparisons show regions with high similarity along with others with an absence of similarity. A schematic diagram of alignments among the *hobo* element from *D. melanogaster* (HFL1), *hobo*-related sequences from *D. sechellia* (*hdelsechi*) and *D. simulans* (*hobo*^{v-a}) as well as a partial sequence of *D. mauritiana* is showed in Figure 4. The similarity level among the different regions of these

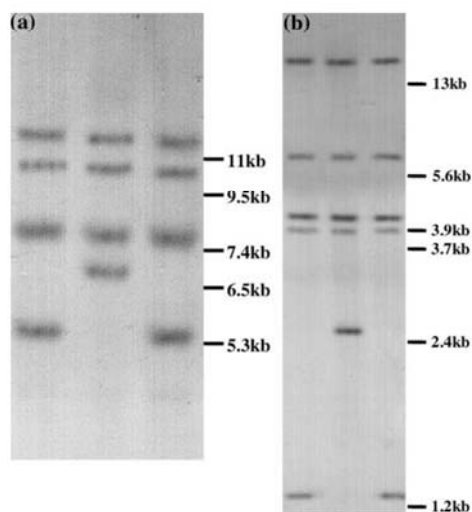


Figure 3. Southern blot analysis from *D. simulans* *white* mutant probed with *white* gene. Genomic DNA from strains of flies wild-type to *white* locus (*Eldorado* and *lozenge*) and mutant *white* of *D. simulans* was digested with (a) *Bgl*II and (b) *Sac*I and probed with the pCaSpeR-*hs* plasmid.

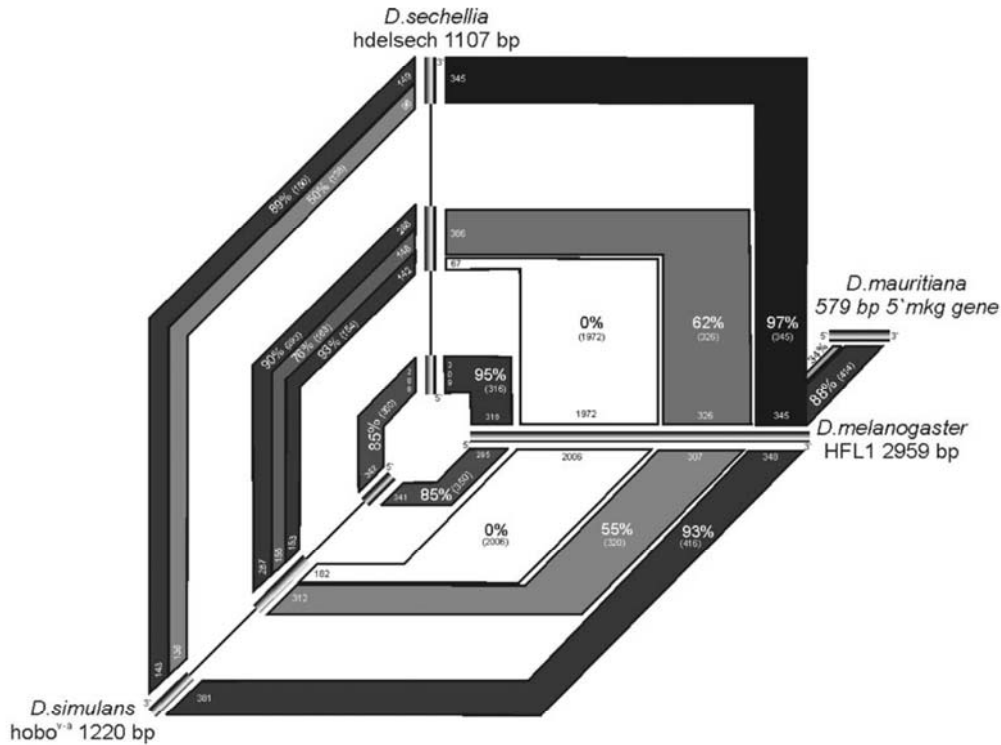


Figure 4. Schematic diagram of alignments among *hobo* elements from *D. melanogaster* (HFL1), *D. sechellia* (*hdelsech*) and *D. simulans* (*hobo^{v-a}*) and a partial sequence of *mkg* gene from *D. mauritiana*. HFL1 is presented as an entire bar; *hdelsech* and *hobo^{v-a}* are presented as discontinuous bars. Both gray scale and the percentage number indicate degrees of identity between these sequences. Numbers in parentheses indicate the size of fragment comparative between two sequences. Small numbers in the tip of each band of comparison represent number of nucleotides of each sequence present inside of the fragment considered.

elements can be compared. When *hobo^{v-a}* is compared to the canonical *hobo* HFL1, disregarding the indels, a general similarity of 81.8% is found. However, the level of shared similarities change along the sequence. In the 5' end, a region with 350 bp presents a similarity of 85% and in the 3' end, three regions can be found, one with approximately 400 bp at the extreme of the 3' end showing a similarity of 93% and in the inner region, 320 bp shows a lower similarity (55%) and other part with 182 bp do not show similarity. The middle region of the HFL1 element, which corresponds to ORF1, is absent in *hobo^{v-a}*. Two other peculiar features observed in the *hobo^{v-a}*, when compared with HFL1, is a 68 bp perfect duplication present in the 3'end region and a 48 bp

insertion in the 5'end, which is not similar to any known sequence.

Another characteristic of *hobo^{v-a}* that clearly places it as a "relic" *hobo* is the absence of both *XhoI* sites. So, these sequences will appear in the Southern blots as bands with molecular weights higher than 2.6 kb and this is characteristic of the "relics" elements. Furthermore, when the *hobo^{v-a}* DNA is used as a probe for Southern blot assays with genomic DNA *XhoI* digested, the characteristic 2.6 bands of canonical *hobo* became faint and the bands with higher molecular weights became darker (data not shown).

A remarkable feature of *hobo^{v-a}* is a great structural similarity with the *D. sechellia* short *hobo* element (*hdelsech*) described by Periquet et al.

(1994). This element has 1107 bp in length and shows a general similarity of 87% with *hobo*^{v-a}, excluding indels. The 5' ITR is identical in both sequences and the 3' ITR differs in two nucleotides. Besides the indels observed in the comparison of these sequences, an elevated similarity is found in the 5' and 3' ends. In the inner sequences similarity decreases to about 70% in some regions (Figure 4). However, a long AT-rich stretch is shared by both sequences. In a general view, it can be supposed that the ancestor of these species shared a common sequence that subsequently diverged to *hobo*^{v-a} and *hdelsech*.

A nr BLAST search using *hobo*^{v-a} as query pointed out, besides the previously mentioned sequences (from *D. melanogaster* and *D. sechellia*), the existence of homologous sequences in *D. mauritiana*. A partial sequence of 579 bp long with a high similarity to the 3' region of *hobo*^{v-a} and *hdelsech* was observed in the tip of one cosmid that contains the *mgk* gene described by Wang, Yu and Long (2004).

Discussion

The *hobo* "relic" sequences so far characterized were supposed to be non-mobilizable (Periquet et al., 1994; Simmons et al., 1998; Galindo et al., 2001; Boussy & Itoh, 2004). In the present work, for the first time, a *hobo*-related sequence was clearly demonstrated to be mobilizable. It was possible for accompanying a hypermutable strain since its establishment in the laboratory, allowing us to detect the moment in that the mutant appeared in the population. The molecular characterization of the particular *hobo*-related sequence (*hobo*^{v-a}) responsible for that mutation, clearly shows that this element is unable to produce the enzymes necessary to its own mobilization. Therefore, it opens the question about the origin of the transposase required to that process. The first candidate as the enzymatic source to mobilization is the canonical *hobo*. Strong evidence point to a recent invasion of the *D. simulans* genome by the canonical *hobo* element (Boussy and Daniels, 1991; Simmons, 1992). Thus, the genome of this species contains active copies of *hobo*. In these circumstances, the enzymes produced by the canonical *hobo* could also mobilize some of the "relic" *hobo*.

The hypothesis that canonical *hobo* is maintained active in some *D. simulans* populations, including the hypermutable strain that gave origin to the *white* mutant (Dswhm), was invigorated from the observation in Loreto, Zaha and Valente (1998) that South American *D. simulans* populations were possibly the last ones to suffer the invasion of their genomes by the canonical *hobo*. This proposition is substantiated by findings described by Boussy and Daniels (1991) in an analysis by Southern Blot of 27 strains collected in different places of the world. From those samples, only four strains were empty of complete canonical *hobo* (E), one from unknown origin and three old strains collected in South America. All other strains, originated from North America, Australia, Europe and South Africa, showed a very strong hybridization signal corresponding to the complete *hobo* element, as well as a 0.7 kb band corresponding to an internally deleted element of 1.1 kb. Loreto, Zaha and Valente (1998) increased the number of South American populations analyzed and found two other populations with faint signals corresponding to the complete *hobo* element and, interestingly, all populations analyzed were devoid of the 1.1 kb deleted element, always present in the samples from other parts of the world, as showed by Boussy and Daniels (1991). Engels (1989) has suggested that deleted elements do accumulate in the genome as a function of time after invasion. On the other hand, Periquet et al. (1989a) propose the hypothesis that the deleted 1.1 kb *hobo* element is probably involved in the regulation of *hobo* activity as a repressor. So, if one of the regulators are absent in South American populations, and some populations are devoid of the complete element and others carry this element, we have the expected conditions for mobilization to take place, including circumstances in which hybrid dysgenesis can occur.

The hypermutable strain that gave origin to the *white* mutant (Dswhm) further derived other sub-strains (Loreto et al., 1998b). One of these sub-strains is almost devoid of complete *hobo* elements, having only a faint band corresponding to a complete element (*dpp*-like mutant strain), although other sub-strains show a strong band corresponding to a complete *hobo* element (Loreto, Zaha and Valente, 1998). As stated above, these conditions point to the possible activity of the *hobo* element in this strain. Other fact that suggests that

hobo^{v-a} was mobilized by the canonical *hobo* transposase is the duplication of 8 bp in the genomic target sequence, since it is a characteristic of *hobo* mobilization (Blackman et al., 1989). Mullins, Rio and Rubin (1989) suggested that the ITRs and the flanking internal regions are the most important ones for Class II transposon mobilization. This fact was well characterized after the development of germ line transformation using the *P* element (Rubin and Spradling, 1982) and *hobo* (Blackman et al., 1989). In this methodology, the transformation vector is constructed using the transposable element ITRs and flanking regions and changing the inner region of the transposon by exogenous DNA. At a first glance, the *hobo*^{v-a} is able to be mobilized by canonical *hobo* transposase, because the structural features required for mobilization, as well as the ITRs and flanking regions, are conserved.

However, other transposase sources to *hobo*^{v-a} mobilization could exist in the *D. simulans* genome. Sundararajan, Atkinson and O'Brochta (1999) shown in *D. melanogaster* a cross-mobilization of *hobo* by *Hermes* element (a related element). Moreover, *hobo* excision events have been demonstrated by Handler and Gomez (1996) in different species of tephritids, which do not have copies of the canonical element. Thus, even though other sources of transposase to *hobo*^{v-a} cannot be discarded at this moment, we suggest that the most probable source is the canonical *hobo* element.

As showed in the results, the *hobo*^{v-a} from *D. simulans* shares a significant level of similarity with the *D. sechellia* short *hobo* element (*hdelsech*) described by Periquet et al. (1994). The ends of both elements are highly conserved and the inner sequences, although showing a small level of similarity, present a good alignment. Therefore, judging by the conservation of ITRs and flanking regions, we may suppose that *hdelsech* is mobilizable in the *D. sechellia* genome. Lachaise and Silvain (2004) estimated as 0.8 MYR the time of divergence between *Drosophila sechellia* and *D. simulans*. The nucleotide divergence estimated between *hobo*^{v-a} and *hdelsech* is entirely compatible with that estimated time of divergence. However, if *hdelsech* is also an mobilizable element, this observation becomes very interesting because it could be an example that a "relic" transposon can stay mobilizable for a relatively long time.

Boussy and Daniels (1991) proposed that *hobo* sequences distribution in *D. melanogaster* and its sibling species could be explained for two different *hobo* introductions: the first one before the divergence of the subgroups *melanogaster* and *montium*; and the second later, in the ancestor of the *melanogaster* complex. Boussy and Itoh (2004) suggested a third very recent invasions into the *melanogaster* complex, corresponding to the canonical *hobo* invasion. Thus, if *hobo* related sequences, as *hobo*^{v-a} and *hdelsech*, are "relics" from the first or second invasion, we could expect that some related sequences might be present in the *D. melanogaster* genome, because the invasion occurred before the divergence of those three species. However, a BLAST search for similar sequences to *hobo*^{v-a} in the *D. melanogaster* genome did not reveal any *hobo* related sequence with structural similarity to *hobo*^{v-a}. Nevertheless, a possible justification for this could be the loss of these sequences in the branch that gave origin to *D. melanogaster*.

Lerat, Rizzon and Biémont (2003) did an extensive analysis of transposable elements of the *D. melanogaster* genome and found remarkable sequence homogeneity among transposable element copies from each family analyzed. The authors point out the high turnover of transposable element copies in the genome as a possible explanation for those findings. This turnover could be a product of natural selection acting against the deleterious effect of transposable elements using chromosome recombination to "clean" the genome from these elements. In this case, just active transposable elements or those inserted in regions with low recombination frequency, as pericentric regions, would survive. The active elements survive because they increase copy numbers above that of the transposons missing. In this context, it is interesting to consider that some "relic" elements could be kept in the genomes by staying mobilizable. The *hobo*^{v-a} and the *hdelsech* could be examples of "relics" that stay mobilizable to escape natural selection.

If the fate of the transposable elements is to be eliminated by high turnover or suffer a slow death by sequence degeneration, after they reach a region of low recombination rates, the only alternative for a long survival is to stay mobilizable. To get that purpose, some interactions between members of different families as well subclasses of

transposable elements are known. These interactions make transposable elements unable to produce the enzymes needed for mobilization, able to transposition using the enzymes made by other transposons. For examples, SINEs employ the LINEs enzymes and the MITES probably use enzymes produced by some class II elements. Moreover, judging by the number of copies in some genomes, SINEs and MITES use those processes very effectively to escape the purifying process of natural selection. We suggested that some *hobo* "relics" could use enzymes produced by other sources to keep themselves active and to avoid natural selection.

Acknowledgments

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CAPÍTULO 3

Dynamics of *hobo*-related sequences in natural populations of *Drosophila simulans*

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Abstract

The *hobo* transposable element can be present under three forms in the *Drosophila* genome: as a complete element (also called canonical), as internally deleted copies, or as *hobo*-related sequences (relics). Some evidence indicated that canonical elements and internally deleted copies are recent acquisitions of *Drosophila* genomes, while the “relics” are old components, normally degenerated, non-functional and until recently considered probably immobile. Much efforts have been done to understand the true relationship of these last sequences with its modern relatives. The analysis of these types of sequences can help us to understand the evolutionary history of these transposable element family. In the present work we performed an analysis with the *hobo*^{v-a} element - a *hobo*-related sequence previously described as mobilizable - in some South American populations of *D. simulans* in order to determine more about its copy number, integrity and genomic stability in compare to the canonical *hobo*, as well how is conserved its sequences in these genomes. We showed that *hobo*^{v-a} seems to constitute a well conserved family of *hobo*-related sequences, which members are clearly defectives but likely still mobilizables. Moreover, the high polymorphism of insertion sites of these sequences observed in the populations suggests that they are active or were active until very recently. Some questions about transposase source to

its mobilization, as well dynamics of maintenance and its similarity to MITEs elements are also discussed.

Key words: transposable elements, *hobo*-related sequences, *D. simulans*, canonical *hobo*

Introduction

Since the first characterization of the *hobo* element family in *Drosophila*, a typical heterogeneous collection of light bands of hybridization to this element, named *hobo*-related sequences, has been observed in the genome of some species. This has been the reason of some speculations about its origin, dynamics of maintenance and its true relationship with its modern relatives. The analysis of such types of sequences, besides helping us to understand the dynamics of evolution of this transposable element family, can help us to shed more light into the evolutionary history of the transposable element as a whole.

The *hobo* transposable element is a Class II DNA transposon (Finnegan, 1989) with short inverted terminal repeats - ITRs- (MCGinnis, Shermoen & Beckendorf, 1983; Streck, MacGaffey & Beckendorf, 1986). The canonical *hobo* (HFL1), isolated from *D. melanogaster*, has ITRs of 12 bp and encodes a transposase (Calvi et al. 1991). Insertion sites of *hobo* elements are typically flanked by an 8 bp duplication of host sequence. The *hobo* element has one of the narrowest distributions of any *Drosophila* transposon so far examined, having been detected only in the *melanogaster* and *montium* species subgroups of the *melanogaster* group (Daniels, Chovnick & Boussy, 1990) and in some strains of *D. willistoni*, which belongs to the other Sophophora group (Loreto et al., 1998a) within the genus *Drosophila*. Of

these, only the *melanogaster* sibling species (*D. melanogaster*, *D. simulans* and *D. mauritiana*) contain large numbers of elements, and only a subset of those are expected to be active. Out of *Drosophila* genus *hobo*-like elements have been found in different species of Tephritidae (Handler & Gomez, 1996; Torti et al. 2005) and in some species of Lepidoptera (DeVault & Narang, 1994; Borsati, Azzoni & Mandrioli, 2003).

The behavior of *hobo* elements in the *D. melanogaster* genome and in its sibling *D. simulans* shares some similarities at the molecular level, in the types of presented sequences and in the geographical and temporal distribution patterns. By Southern blot, using *XhoI* *hobo* restriction fragment (Streck et al, 1986; Blackman et al, 1989) as a indicator of integrity, three different types of *hobo* elements can be distinguished in the *D. melanogaster* genomes: full-size *hobo* elements characterized by a 2.6kb fragment, typical internally deleted elements whose *XhoI* restriction fragment is less than 2.6kb, and elements giving high molecular weight bands (more than 2.6kb) also called *hobo*-related sequences or “relics”. Surveys of *D. melanogaster* stocks from different locations and dates of capture (Streck, MacGaffey and Beckendorf 1986; Periquet et al. 1989a, 1989b) revealed that stocks captured before the 1960s lack canonical *hobo* elements (E strains), while the more recent ones contain complete and defective *hobo* elements (H strains). These findings suggest that this element has been recently introduced into this

species by horizontal transfer (Periquet et al. 1989_a; 1989_b; 1990; Boussy & Daniels, 1991; Pascual & Periquet, 1991).

The *hobo* element is also found in *D. simulans* under three forms (Streck, MacGaffey & Beckendorf, 1986; Boussy & Daniels, 1991). Simmons (1992) showed that most of a full-size *hobo* from *D. simulans* is virtually identical in DNA sequence to the canonical *hobo* of *D. melanogaster*, differing only at three positions out of 2563 sequenced (the internal *Xho*I fragment). Complete elements in *D. simulans* are approximately 3kb in length and typical deleted elements are also present, *h del sim* (1080 bp) being the most frequent one (Boussy & Daniels, 1991). Besides carrying full-size and smaller *hobo* elements, *D. simulans* also carries many copies of *hobo*-related sequences detected by Southern blot as for *D. melanogaster*. The temporal and geographical distribution patterns of *hobo* in *D. simulans* also show that all recently collected strains contain complete *hobo* element (H strains). However, the pattern for strains collected before 1950 is not so clear as for *D. melanogaster*. Some evidences point to a recent invasion of *D. simulans* genome by the *hobo* elements in South American populations. Boussy & Daniels (1991) performed an analysis of 27 strains of *D. simulans* and found only 4 empty for complete *hobo* (E strains), one from unknown origin and three old strains collected in South America. Loreto et al., (1998c) increased the number of *D. simulans* South American populations analyzed and found a few older strains of laboratory with faint signals corresponding to the

complete *hobo* element. Moreover, interestingly, all populations analyzed were devoid of the 1.1kb typical deleted element, always present in the strains from other parts of the world (Boussy & Daniels, 1991). These findings reinforce the hypothesis of the recent invasion in South American populations.

A curious feature of the *D. melanogaster* and *D. simulans* genome is that only H strains show complete and species-specific defective *hobo* elements, but both E and H strains show *hobo*-related sequences that yield a different restriction pattern of weaker bands in Southern blot analyses. Pascual and Periquet (1991) suggested that these sequences might be the remnants of a putative relic *hobo* element originated from earlier introductions. More recently some effort has been done to characterize these *hobo* “relics” in an attempt to understand its role in the evolutionary history of *hobo* family. Simmons et al. (1998) and Galindo et al. (2001) described *D. melanogaster* *hobo*-related sequences as non-functional and normally degenerated. These sequences showed various re-arrangements in comparison to HFL1 and, moreover, presented numerous indels and substitutions. The average degree of sequence identity of these elements to HFL1 in homologous regions was approximately 85% (range: 80-92%). These authors suggested these *hobo* “relics” are immobile and distantly related to modern *hobo*. Boussy & Itoh (2004) have characterized another *hobo*-related sequence from the genome of *D. melanogaster*. This sequence was shown to

be clearly non-functional and probably incapable to transpose in the presence of a functional transposase. Its 3' 12 bp ITR is intact but the 5' ITR is, in contrast, extremely degenerated. Thus, most of *hobo*-related sequences described were degenerated, non-functional and considered as probably immobile elements. However, Torres et al. (2005) detected a *hobo*-related sequence – *hobo*^{v-a} in the genome of a hypermutable strain of *D. simulans* whose insertion into the *white* locus raised a *de novo white* mutation. It was the first *hobo* “relic” showed to be able to mobilize. It is a shorter *hobo* related element (1220pb) presenting, overall, roughly 18% of divergence to the canonical *hobo*. This relic is a clearly defective element because it has many indels and none functional ORFs. However, its ITRs and flanking regions are extremely conserved. Because this *hobo* relic is a defective element but kept the regions required for its mobilization well conserved and has been detected raising a *de novo* mutation event, the authors suggest that this sequence could have been mobilized *in trans* by the canonical element. In the present work we analyzed this *hobo*^{v-a} element in some South American populations of *D. simulans* in order to determine more about its copy number, integrity and genomic stability in comparison to the canonical *hobo*, as well how conserved are its sequences in these genomes.

Materials and methods

Strains

The following strains were employed in the present study:

Eldorado (ELD) – collected in Eldorado do Sul, Rio Grande do Sul, Brazil, in 1990

dpp, lz, rb, zp, white - originated from a hypermutable strain of *D. simulans* described by Loreto et al. (1998b)

Itapuã - collected in Viamão, Rio Grande do Sul, Brazil, in 2002

yellow – derived from a spontaneous mutant of the *yellow* locus encountered in a population sample from Itapuã, Rio Grande do Sul, Brazil, in 1982

Campeche – collected in Ilha do Campeche, Santa Catarina, Brazil, in 2002

Lagoa – collected in Morro da Lagoa, Florianópolis, Santa Catarina, Brazil, in 2003

S4 – collected in Florianópolis, Santa Catarina, Brazil, in 1997

Mirassol – collected in Mirassol, São Paulo, Brazil, in 2002

SPF3 – collected in Paulo de Faria, São Paulo, Brazil, in 2005

Arraial – collected in Arraial do Cabo, Rio de Janeiro, Brazil, in 2004

Agronomia - collected in Montevideo, Uruguay, in 2002

Farrapos – collected in Rio Negro, Uruguay, in 2002

Dom Bosco – collected in Rocha, Uruguay, in 2002
Solis I e II – collected in Maldonado, Uruguay, in 2002
Castillos – collected in Rocha, Uruguay, in 2002
Chile – collected in La Florida, Chile, in 1992
Peru – originally collected in Lima, Peru, in 1956 (obtained from Bowling Green Center n° 14021-0251-5)
Zarate – collected in Zarate, Argentina, in 2004
Line2 – collected in Winters, CA, USA, in 1992

Southern blot analysis

Genomic DNA was prepared from approximately 100 adult flies according to Jowett (1986). DNA samples were digested with restriction enzymes following manufacturer directions (Invitrogen). The employed enzymes were *Bgl*III and *Xho*I according to the restriction map of the *hobo* element (Calvi et al, 1991) in order to investigate the copy number and the integrity of *hobo* elements, respectively. *Bgl*III does not cut the *hobo* element and produces fragments of different lengths that can be derived from relic, deleted or full-size elements. Given the difficulty in separating the bands corresponding to the canonical and relic copies only by *Bgl*III pattern we also analyzed the autoradiographies of the *Xho*I digests probed with *hobo*^{v-a} element counting bands higher than 3 kb to estimate the relics copy number. *Xho*I cuts a full-sized *hobo* at two sites close to each end producing an

internal fragment with about 2.6 kb, and shorter fragments from deleted *hobo*.

DNA fragments were separated by electrophoresis on 1% agarose gels and transferred to nylon membranes (Hybond N+/GE Healthcare). The membranes were hybridized to a random primer-labeled probe at 60°C in 5×SSC, 0.1% SDS, 5% dextran sulfate, and 20-fold dilution of liquid block. The filters were washed twice with 0.2×SSC and 0.5% SDS for 15 min at 60°C. Hybridization and detection were performed using a Gene Images Kit (GE Healthcare) according to manufacturer instructions. As probes for *hobo* element we employed two plasmids: 1) the *pHX4* plasmid which contains a 2.6 kb of *hobo* element of *D. melanogaster* (the 2.6 kb fragment was removed from a complete element contained into the *pHFL1* plasmid); 2) the *pHVA* plasmid which contains the 0.8 kb internal fragment of *hobo*^{v-a} element cloned into the *pCR4- TOPO*® plasmid (Invitrogen) (Torres et al, (2005), accession number AY764286).

DNA amplification and sequencing

PCR reactions were performed in 25µl volumes using approximately 25ng of template DNA, 20pmol of each primer, 1.5mM of MgCl₂, 50µM of each nucleotide and 1U of Taq DNA Polymerase Recombinant (Invitrogen) in 1× PCR buffer. After an initial denaturation step for 2min at 95°C, 35 cycles consisting of 40s denaturation at 95°C, 40s annealing at 55°C and 1min

extension at 72°C were carried out. An additional extension step of 5 min at 72°C was performed after the last cycle. The primers used ($h^{v-a}1s$: 5'-CATAACGGAAGGGTAGAGAAG-3' and $h^{v-a}2as$: 5'-CGTCCACCCGATAAACTC-3') anneal into the *hobo^{v-a}* sequence isolated from *D. simulans* at positions 200 to 219 and 1169 to 1188 respectively. The amplified fragment of each strain was separated by electrophoresis on 1% agarose gel. The PCR products were purified and cloned into pCR4-TOPO® plasmid (Invitrogen). DNA sequencing was performed directly from the purified plasmids (2 to 3 clones selected) in a MegaBACE 500 automatic sequencer. The dideoxy chain-termination reaction was implemented with the use of the DYEnamic ET® kit (GE Healthcare), and M13 primers for sequencing. The sequences were then inspected and submitted to a “confidence consensus” analysis using the Staden Package Gap 4 program (Staden, 1996). The sequences so obtained were aligned using the ClustalX 1.81 program (Jeanmougin et al, 1998) according to the system default parameters. The phylogenetics analyses were executed using the neighbor joining method (NJ) (Saitou and Nei, 1987) according to the Kimura two-parameter model (Kimura, 1980) using Mega 2.1 (Kumar et al 2001). BLAST analyses were performed using search engines at the Berkeley *Drosophila* Genome Project (BDGP; www.fruitfly.com) and GenBank (www.ncbi.nlm.nih.gov) to look for sequences with general similarity.

IRAP-like analysis (ITAP)

A technical IRAP-like (Inter-Retrotransposon Amplified Polymorphism) was performed to analyze the stability of both *hobo* canonical and *hobo*^{v-a} sequence in the genome of some *D. simulans* strains. The original method was described for retrotransposons (Kalendar et al., 1999; Schulman et al., 2004). It detects two retrotransposons or LTRs sufficiently close to one another in the genome to permit PCR amplification of the intervening region. However, for this method to be successful the element under study must present some features as following: structurally, it must contain defined and conserved sequences which can be used for cloning of specific markers and flanking sequences. Secondly, replicationally active members of a (retro)transposon family will produce new insertions in the genome leading to polymorphism that may be detected. The element must have a high copy number and these copies must be dispersed in the genome. This method requires only intact genomic DNA as template and PCR reagents and apparatus for amplification. There are no restriction enzyme digestion or adapter ligation steps. In our case, because this method has been adapted for a transposon family, we will describe it as ITAP (Inter-Transposon Amplified Polymorphism).

For the ITAP analysis we have designed primers facing outward from the sub-terminal regions of the *hobo* canonical and *hobo*^{v-a} transposons. Primers were designed to match the regions next to the 5' and 3' ITRs of the

hobo canonical element (accession M69216) and *hobo*^{v-a} (accession AY764286). For the *hobo* canonical the reverse primer is complementary to bases 54-73, and the forward primer matches bases 2909-2928. For the *hobo*^{v-a} the reverse primer is complementary to bases 43-61, and the forward primer matches bases 1169-1188. The forward primer anneal to a conserved region between both elements.

The ITAP PCR was performed in a 25µl reaction mixture containing 25ng DNA, 1x PCR buffer, 1.5mM MgCl₂, 10 pmol each primer, 0.2 mM dNTP, 1U Taq DNA Polymerase Recombinant (Invitrogen). Amplification was performed in a PTC-100 (MJ Research) in a 0.2 ml tubes. The PCR reaction program consisted of: 1 initial denaturation step for 2min at 95°C, 35 cycles consisting of 40s denaturation at 95°C, 40s annealing at 55°C and 2min extension at 72°C. An additional extension step of 5 min at 72°C was performed after the last cycle. Products were analyzed by electrophoresis on 1.5% agarose gel (Invitrogen) and detected by ethidium bromide staining.

Results

Analysis of hobo copy number by Southern Blot

The *hobo* canonical and *hobo*^{v-a} element copy number in some *D. simulans* strains was estimated through Southern blot analysis. The *Bgl*III digests showed that the total number of *hobo* elements is variable among the

studied *D. simulans* strains (Figures 1 and 2 in Appendix 2). The values of copy number for canonical and relic *hobo* elements in each strain are presented in the Figure 1.

The comparative analysis of integrity of both *hobo* sequences (canonical and *hobo^{v-a}*) in the genome reveal a peculiar pattern for each sequence (Figures 2 and 3). Genomic DNA digested with *XhoI* when probed with *pHX4* plasmid (canonical *hobo* probe) present the typical pattern of full-sized *hobo* elements in the genome: a remarkable fragment of approximately 2.6kb corresponding to the complete element, some smaller fragments with almost the same intensity of the 2.6 kb fragment (internally deleted copies) and several other higher fragments with faded hybridization signal which correspond to the relics copies. All strains showed the 2.6 kb fragment expected, with variable signal intensities. The yellow and *dpp* strains presented a very weak signal for this fragment, indicating that these strains probably harbor least copies of complete element than other strains analyzed. All the strains carry defective copies with variable sizes and densities. However, excepting Chile and D.Bosco strains, none strain presented the 0.7 kb typical fragment of *D. simulans*, corresponding to the *hdelsim* internally deleted *hobo* element described to other studied world strains.

When we reprobated the filters with *pHVA* (relic element probe) we observe a clearly distinct pattern. The 2.6 kb fragment appears with a

smaller intensity than described above which is observed for the smaller fragments as well. However, the higher fragments (high molecular weight) appear now with more intensity than before and with a considerable variation of sizes and densities inter strains (Boussy & Daniels, 1991).

Analysis of hobo relics sequences

Using primers designed to match the *hobo*^{v-a} sequence, we amplified a fragment of about 1 kb in each studied strain. This amplification product correspond to an internal fragment of *hobo*^{v-a} which includes the central and sub-terminal regions but no ITRs. Products of Goias (GOI), Mirassol (Mir), yellow (yel), dpp, D.Bosco (DB) e Chile (Chi) strains, which are derived from different geographic locations were isolated and sequenced. The sequences overall present the sub-terminal regions better conserved than the central internal portion. This portion is rich in repetitive sequences that correspond to an imperfect TAA microsatellite sequence. We were unable to obtain a whole sequence of the same clone because the overlapping region of reverses and forwards reads are into this microsatelite region and the confidence of sequencing is very low in this portion. The reads number available for each clone (3 to 4) was not enough to solve this problem. This region seems be highly variable among the sequences. The sequences like microsatelites present peculiar characteristics with regard to evolutionary process itself as, for instance, higher mutation rate (Amos, 2001) which could interfere in the

analysis of sequence similarity of sub-terminal sequences. Therefore, only the sub-terminal regions were employed in the further analyses.

The analyzed sequences corresponding to the 5' and 3' sub-terminal regions. For the 5' sub-terminal sequences was observed, in most of the strains, a high similarity among them along roughly 295 bp (Figure 3 in Appendix 2). However, two sequences (from Mirassol and D.Bosco strains) presented higher divergence in relation to the others. The first 145 bp were very similar to other sequences but the next 150 bp correspond to the microsatellite portion and, then, no alignment was possible for this region. The microsatellite region in these sequences should have probably enlarged and overlapped the region of more similarity with other sequences. The values of p distances between the 5' sub-terminal sequences analyzed are present in the Figure 4. The average divergence between the sequences range from 0.15 to 0.27, which would indicate an estimated time of divergence between the sequences of roughly 0.5 a 0.8 MYR. The sequence CHI2, which presents two deletions, showed a higher divergence.

The 3' sub-terminal regions are more conserved than 5' sub-terminal regions (Figure 4 in Appendix 2). All strains present a higher degree of similarity along roughly 360 bp.

The values of p distances between the 3' sub-terminal sequences analyzed are present in the Figure 5. The highest divergence (2.5%) was observed between the sequences of two different clones of the same strain

(dpp, clones 3 and 4). The estimated time of divergence between these sequences is roughly 0.75 MYR, similar that described for the 5' sub-terminal sequences.

The two parts of sub-terminal sequences together corresponded to approximately 65% of the amplified sequence of the transposons. In most of clones from different analyzed strains the similarity observed along this extension was quite high. The exception was only two clones from D.Bosco and Mirassol strains where was observed an increased of microsatellite region in direction to the 5' sub-terminal region. Therefore, the extension of similarity between these sequences and the others was smaller in this region, but between them the similarity was high.

The 5' and 3' sub-terminal sequences aligned were subjected to an independent phylogenetic analysis using the neighbor joining method. For these analyses we include the sequences of *hobo*^{v-a} (AY764286) and *hdelsech* (X77577) (Figure 5 in Appendix 2). The phylogeny of the 5' portion of these sequences (Figure 6) confirms the high similarity between them. Clones of Mirassol and D.Bosco strains were more similar to each other than the other sequences in this region. The *hobo*^{v-a} element and *hdelsech* seems more similar to each other in this region as well.

The phylogenetic analysis for the 3' sub-terminal portion of the sequences described previously (Figure 7) shows a high similarity between them and *hobo*^{v-a}. The trees show still that clones from different strains are more

similar to each other than clones from the same strain suggesting that there are different clusters of *hobo*^{v-a} in the genome of these strains.

Results of ITAP analyses

The ITAP method was employed to examine polymorphism in insertion sites between two canonical - canonical, *hobo*^{v-a} - *hobo*^{v-a} and canonical - *hobo*^{v-a} transposons, regarding the disperse nature of these transposon family in the *D. simulans* genome. We have designed primers facing outward from sub-terminal regions (next to ITRs) of both elements canonical *hobo* and *hobo*^{v-a} from *D. simulans*. These regions are well conserved between the sequences (which was confirmed by sequencing) and they are considered essential for transposition of these elements. The transposons may integrate in principle in either orientation into the genome, and hence any two members of a transposon family may be found head-to-head, tail-to-tail, or head-to-tail. For the first two orientations, a single primer suffices to generate PCR products from elements sufficiently close to one another. To amplify intervening genomic DNA for elements in head-to-tail orientation, we must use both 5' and 3' sub-terminal primers, which in turn should produce some bands from the other orientations as well. Therefore, the number of products visible by ITAP should reflect the number of canonical or *hobo*^{v-a} transposons close enough to one another to permit PCR amplification, which

in turn could be suggestive of the copy number and genomic organization of this transposon family in the *D. simulans* genome.

The observed ITAP pattern was polymorphic between the strains analyzed (Figure 8). A similarity index was constructed to estimate the overall degree of polymorphism in the banding pattern, based on the presence or absence of bands scored. A value of 100 indicates that the patterns are totally identical between the strains compared and a value of 0, total dissimilarity. Using the different primer combinations, amplification products ranging from 300 pb to about 400 pb were generated. The pattern observed between the strains with each primers match tried was quite variable. To canonical-canonical primers match, for instance, it was observed products conserved between most of strains (about 2.1 kb), products shared between some strains (about 0.7 kb), as well products lineage-specific (most of them). This variation in turn could reflect, even if underestimated, the variation in the copy number between the strains. Moreover, the observed pattern indicates the high polymorphism for insertion sites of these canonical copies between the strains. The similarity index to this pattern is presented in Figure 5 in Appendix 2.

The observed pattern with *hobo*^{v-a} - *hobo*^{v-a} primer pair presents products shared between most of strains as well (about 2.8 kb), shared between some strains (about 2.6 kb) and many products lineage-specific. The indicatives of copy number as well the polymorphism to insertion sites are in

accordance with Southern blot results. The similarity index to this pattern is presented in the Figure 6 in Appendix 2.

Because the distribution of canonical and relic copies in the genome may be alternate one to another, a third primers match was employed to examine the polymorphism of insertion sites between both elements. In this match the observed pattern was apparently less variable between the strains than the other primers matches, which may be confirmed by higher similarity index presented in the Figure 7 in Appendix 2.

Although the results of ITAP method presented here have gave good indicatives of the variation in the copy number and the insertion sites to both elements, the amount of polymorphism revealed on the strains level was too great to permit the use of ITAP in interstrain phylogenetic analyses. However, the great polymorphism of insertion sites presented by the canonical *hobo* and *hobo*^{v-a} elements in the different strains, including between the originated isofemale lines, since first isofemale line Dshs (hypermutable strain), suggests that both elements are active in the genomes of these strains or were until a recent past period.

Discussion

So far, most of the *hobo*-related sequences analysis has been limited to its search, isolation and identification in some *D. melanogaster* and *D. simulans* E strains stocks (Simmons et al., 1998; Galindo et al., 2001; Boussy & Itoh, 2004). In the present work, we performed an analysis of *hobo*^{v-a} – a mobilizable *hobo*-related sequence described in Torres et al., (2005) - in natural populations of *D. simulans*. We have shown that *hobo*^{v-a} seems to constitute a well conserved family of *hobo*-related sequences, whose members are clearly defectives but likely still mobilizable. Moreover, their great insertion sites polymorphism observed in the populations suggests that they are active or were active until very recently in these genomes. If these relic sequences still display the potential to mobilization and activity, the question about what is the transposase source used to these functions remains open. If we think about a more recent period, since the last invasion of autonomous *hobo* in the *D. simulans* genome, this element could be the first candidate to serve as a transposase source to the mobilizable relics. In fact, strong evidences point to a recent invasion of the *D. simulans* genome by the canonical *hobo* element (Boussy & Daniels, 1991; Simmons, 1992) and to the presence of active copies of this element. Loreto, Zaha and Valente (1998c) propose that South American *D. simulans* populations were possibly the last ones to suffer the invasion of their genomes by the autonomous

hobo. This proposition was previously substantiated by the findings described by Boussy and Daniels (1991) that found only four strains empty of canonical *hobo* (E strains), one from unknown origin and three from South American origin, in populational samples of *D. simulans* collected in different regions of the world. All other strains, originated from North America, Australia, Europe and South Africa, showed a very strong hybridization signal corresponding to the complete *hobo* element, as well as a 0.7 kb band corresponding to an internally deleted element of 1.1 kb. Nevertheless, all the populations analysed by Loreto, Zaha and Valente (1998c) were devoid of the 1.1 kb deleted element while two other populations presented faint signals corresponding to the complete *hobo* element. The finding of other populations devoid of that deleted element, in the present work, reinforce the hypothesis of a recent invasion. Periquet et al., (1989) propose that typical deleted elements could be involved in the regulation of autonomous *hobo* activity, acting as a repressor. So, we could think that autonomous *hobo* invaded recently the genome of the South American *D. simulans* populations and that it is in the middle of its activity, increasing its copy number and producing transposase. If we still suppose that one of its probable regulators is absent in these populations but, on the other hand, mobilizable relics are present, we have the expected conditions for the produced transposases to mobilize these relics.

However, if we take into account that these relic elements are ancient by nature, probably remnants of earlier invasions of *hobo* in the *Drosophila* genome as proposed by Boussy & Daniels (1991), and that the modern autonomous element invaded the genome only recently, how, then, this relic stayed active during the lack period of the autonomous element? Probably other transposase sources could have been used. The *hobo* transposons belong to a great superfamily of related elements, which share among themselves some structural and functional features with regard to transposase. Moreover, cross-mobilization between *hobo* and *Hermes* have been reported in *D. melanogaster* (Sundararajam, Atkinson and O'Brochta, 1999) as well as the transposition dependent or independent of transposase out of drosophilids has been noted in species that do not have copies of the autonomous element (Handler & Gomez, 1996). So, in those circumstances described above other transposase sources originated from related elements could have served to the relic mobilization.

Pointed the evidences of mobility and activity of *hobo^{v-a}* in the genome as well as the evidences of its ancient presence in *D. simulans*, the question that we raise now is about what is the role (or fate) of these elements in the genome? Taking into account that some features observed in *hobo^{v-a}* are quite similar to those described to MITEs elements, would *hobo^{v-a}* elements be a new *Drosophila* MITEs family or, more interestingly, a sequence heading for a MITE form and, thus, ensuring its maintenance in the genome for a long time

and in a very efficient way? MITEs are characterized as very short (normally less than 600 bp), non-autonomous, Class II elements. While copy numbers of other Class II elements are relatively low, MITEs are often found in high copy numbers in their host genome. They possess ITRs flanked by target site duplications (TSDs) and have been classified into several superfamilies based on the structural features of their ITRs and TSDs (Feschotte et al., 2002). Since MITEs lack coding sequences, their mobilization requires transposase encoded *in trans* by autonomous elements related to specific MITEs. According to a model for the origin of MITEs, a MITE family is composed of MITEs subfamilies that have arisen from related autonomous elements in a single genome, like the conventional non-autonomous elements, but they possess some features that allow them to be subsequently amplified to higher copy numbers than their sibling conventional non-autonomous elements. Based on the similarity between a MITE family and a potential partner, Feschotte et al., (2002) propose different levels of MITEs classification. By the features observed in the *hobo^{v-a}* elements, originally described in Torres et al., (2005), these elements could be considered as MITEs of level 2, once that they share similarity of sequence in the TSDs, ITRs and sub-terminal regions but not in the internal part in relation to the canonical *hobo*. However, judging by the size and copy number, *hobo^{v-a}* does not seem to follow a typical MITE pattern, although a few MITEs higher than 1 kb in length have already been described (MITEs of *Mutator*-like superfamily of *Arabidopsis*

thaliana) as it is the case for MITEs whose copy number is lower than 50 copies (MITEs of *A. thaliana* *hAT* superfamily) (Feschotte et al, 2002). Therefore, although *hobo*^{v-a} elements do not have all typical MITEs features, they share more features with these elements than with conventional non-autonomous elements. So, we suggest that *hobo*^{v-a} elements would be in an intermediate form, in the boundary between *hobo*-related sequence and *hobo* MITE, in a way that they have achieved to escape from genomic inactivation and elimination, staying active (or mobilizable) for a long time.

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Figure legends

Figure 1 – Graphic representing the copy number of the *hobo* canonical (dark bars) and *hobo*^{v-a} (light bars) elements in some strains of *D. simulans* (see Material and Methods). The mean value of copy number canonical is 20.11 ± 2.08 and the *hoboVA* is 16.79 ± 2.7

Figure 2 – Southern blot analysis of genomic DNA of some strains of *D. simulans* (see Materials and methods) digested with *Xho*I and probed with **A)** *pHX4* plasmid (***hobo* canonical probe**) and **B)** *pHVA* plasmid (***hobo* relic probe**).

Figure 3 – Southern blot analysis of genomic DNA of some strains of *D. simulans* (see Materials and methods) digested with *Xho*I and probed with **A)** *pHX4* plasmid (***hobo* canonical probe**) and **B)** *pHVA* plasmid (***hobo* relic probe**).

Figure 4 – Matrix of distance p between the 5' sub-terminal sequences isolated from some clones in different strains. Numbers close to the names indicate different clones of the same strain. Numbers in parentheses are the standards errors.

Figure 5 – Matrix of distance p between the 3' sub-terminal sequences isolated from some clones in different strains. Numbers close to the names indicate different clones of the same strain. Numbers in parentheses are the standard errors.

Figure 6 – Neighbour joining K-2P phylogenetic model for sequences of 5' sub-terminal region of the *hobo* elements isolated from different strains of *D. simulans* and the equivalent region in *hobo^{v-a}* and *hdelsech*. Bootstrap values are indicated above the internal branches .

Figure 7 – Neighbour joining K-2P phylogenetic model for sequences of 3' sub-terminal region of the *hobo* elements isolated from different strains of *D. simulans* and the equivalent region in *hobo^{v-a}* and *hdelsech*. Bootstrap values are indicated above the internal branches .

Figure 8 – Banding patterns from some strains of *D. simulans* generated by ITAP. **A)** With canonical primers match; **B)** with *hobo^{v-a}* primers match and **C)** canonical-*hobo^{v-a}* primers match. The order in each gel is : 1- rb; 2 - dpp; 3 - w; 4 - lz; 5- yell; 6 - Eld; 7 - Peru; 8 - Zarate; 9 - Soll; 10 - Mirassol; 11 - Arraial; 12 - Camp; 13 - Line2; 14 - Lag; 15 - Cast; 16 - Agro; 17 - Farr; 18 - Chile. Marker sizes 1 kb Plus DNA Ladder are shown on the left side of each gel before the strains.

Figures

Figure 1

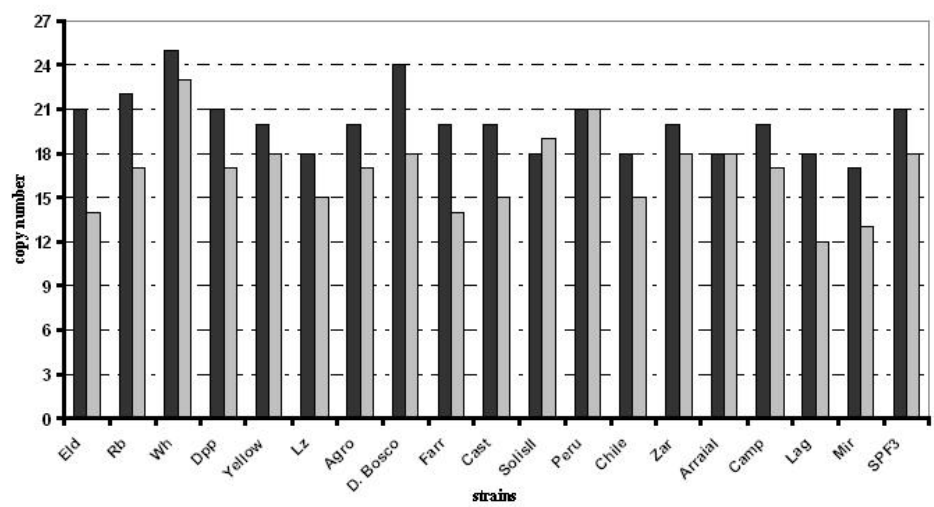


Figure 2

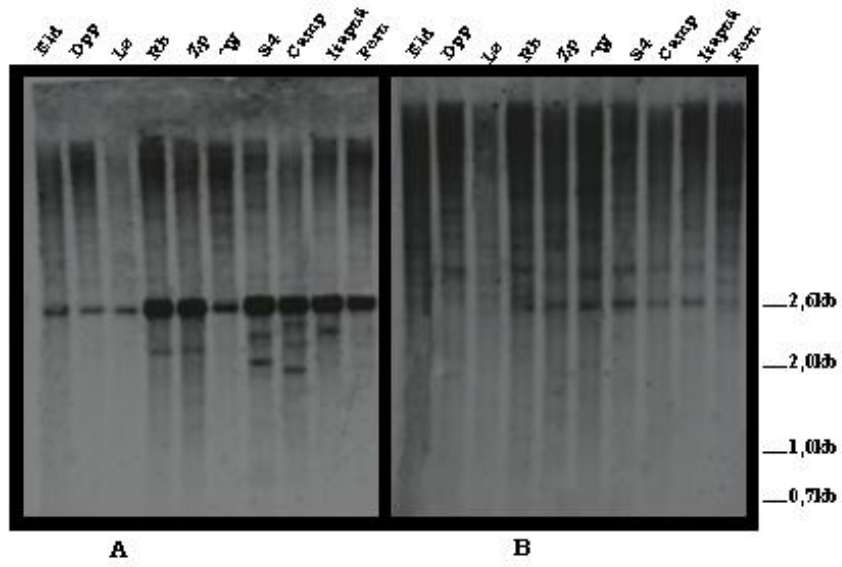


Figure 3

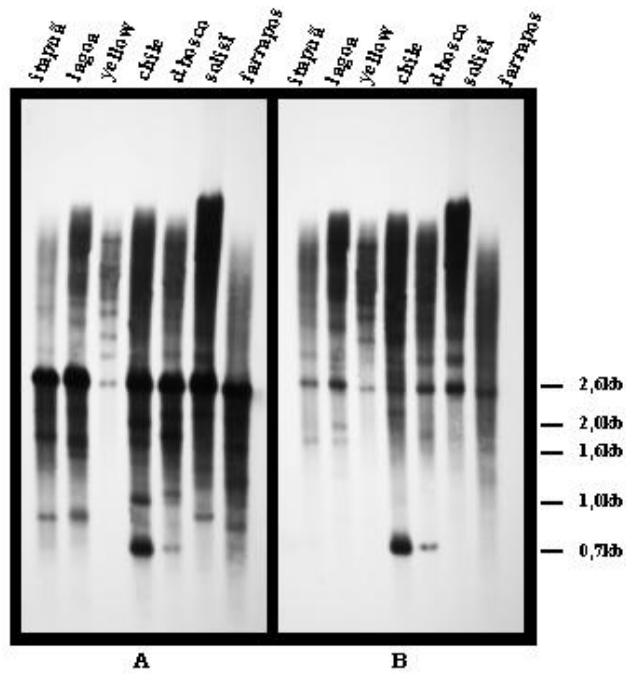


Figure 4

	CHI3	YEL1	MIR4	MIR5	GOI2	GOI1	MIR1	YEL2	CHI2
CHI3		[0.007]	[0.008]	[0.007]	[0.007]	[0.007]	[0.006]	[0.009]	[0.014]
YEL1	0.015		[0.009]	[0.007]	[0.005]	[0.007]	[0.007]	[0.009]	[0.013]
MIR4	0.022	0.024		[0.005]	[0.008]	[0.008]	[0.006]	[0.008]	[0.014]
MIR5	0.015	0.017	0.007		[0.006]	[0.007]	[0.005]	[0.007]	[0.013]
GOI2	0.015	0.007	0.021	0.014		[0.005]	[0.006]	[0.008]	[0.013]
GOI1	0.018	0.017	0.024	0.017	0.010		[0.007]	[0.009]	[0.014]
MIR1	0.015	0.017	0.014	0.007	0.014	0.017		[0.007]	[0.012]
YEL2	0.026	0.027	0.024	0.017	0.024	0.027	0.017		[0.014]
CHI2	0.069	0.064	0.067	0.060	0.060	0.074	0.053	0.064	

Figure 5

[DPP6	YEL2	DPP3	Db1	DPP4	GOI1	DPP2	GOI2	CHI2	YEL1]
DPP6		[0.005]	[0.006]	[0.004]	[0.006]	[0.003]	[0.003]	[0.004]	[0.004]	[0.004]
YEL2	0.011		[0.007]	[0.005]	[0.006]	[0.005]	[0.005]	[0.005]	[0.005]	[0.005]
DPP3	0.017	0.019		[0.007]	[0.008]	[0.006]	[0.006]	[0.007]	[0.007]	[0.007]
Db1	0.006	0.011	0.017		[0.006]	[0.003]	[0.003]	[0.004]	[0.004]	[0.004]
DPP4	0.014	0.014	0.025	0.011		[0.006]	[0.006]	[0.006]	[0.006]	[0.006]
GOI1	0.003	0.008	0.014	0.003	0.011		[0.000]	[0.003]	[0.003]	[0.003]
DPP2	0.003	0.008	0.014	0.003	0.011	0.000		[0.003]	[0.003]	[0.003]
GOI2	0.006	0.011	0.017	0.006	0.014	0.003	0.003		[0.004]	[0.004]
CHI2	0.006	0.011	0.017	0.006	0.014	0.003	0.003	0.006		[0.004]
YEL1	0.006	0.011	0.017	0.006	0.014	0.003	0.003	0.006	0.006	

Figure 6

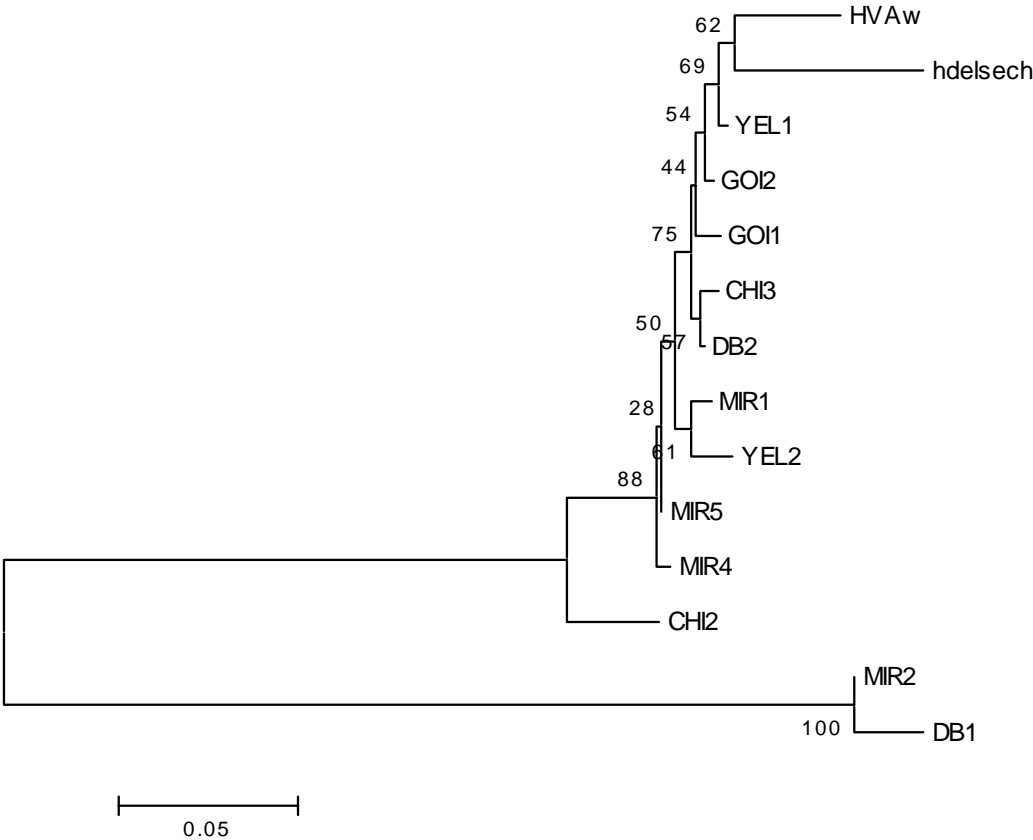


Figure 7

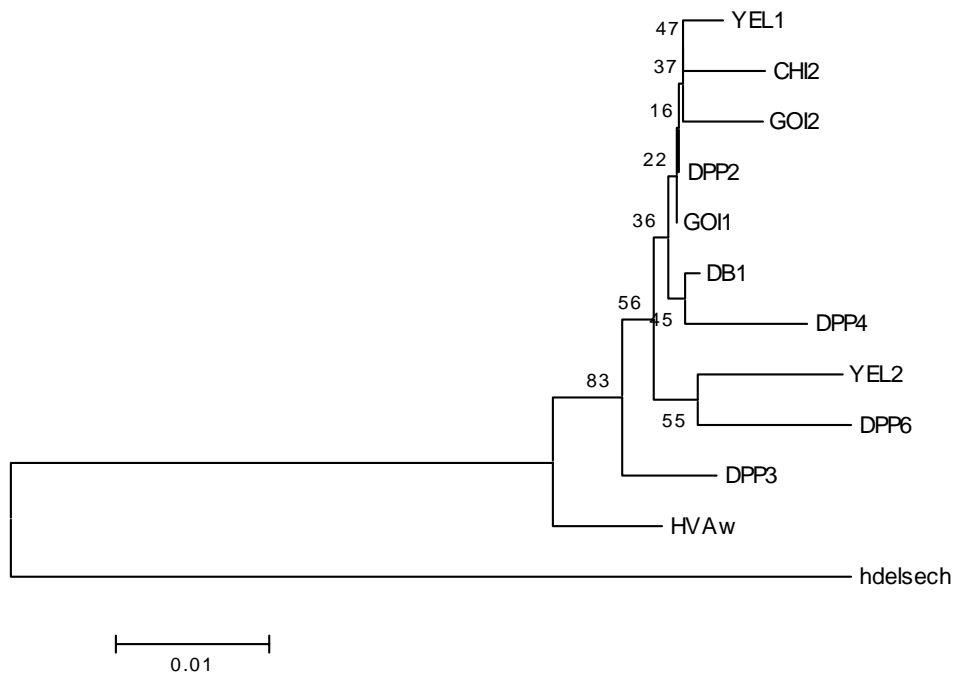
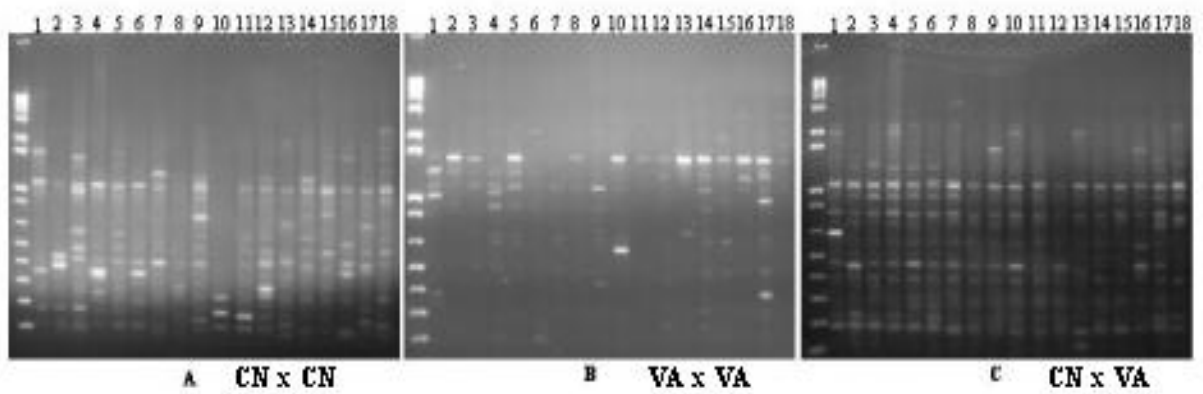


Figure 8



CAPÍTULO 4

DISCUSSÃO GERAL, CONCLUSÕES E PERSPECTIVAS

Seqüências relacionadas a *hobo*, desde suas descobertas, têm gerado instigantes discussões em torno do seu verdadeiro relacionamento com a família *hobo* de elementos transponíveis, em função do seu padrão bastante peculiar, observado em estudos de hibridizações com esse elemento. Da mesma forma, questões sobre suas origens e sua dinâmica de manutenção no genoma têm sido levantadas. Dentre algumas possibilidades de relacionamento com elementos funcionais, a mais fortemente aceita é a de que essas seqüências são remanescentes de elementos autônomos (também referidas como “relics”), que invadiram o genoma há muito tempo, mas atualmente estão inativos. Assim, essas seqüências “relics”, atualmente, podem existir isoladamente num genoma ou juntamente com elementos autônomos oriundos de invasões recentes.

Estudos de algumas seqüências relacionadas a *hobo* têm revelado divergências esperadas em relação ao elemento autônomo, defectividade e, principalmente, imobilidade dessas seqüências. O presente trabalho é uma contribuição ao entendimento da dinâmica de seqüências relacionadas a

hobo e seu papel na evolução dessa família de elementos transponíveis. A oportunidade de estudar esse tipo de seqüência surgiu da nossa necessidade de caracterizar o agente causador de uma mutação *de novo* no gene *white* em uma linhagem hipermutável de *D. simulans* (Loreto *et al.*, 1998b; Torres, 2001). Para nossa surpresa, esse agente foi um elemento (ou seqüência) relacionado a *hobo* (Torres *et al.*, 2005) uma vez que, até então, estes tipos de elementos eram considerados imóveis no genoma. A partir dessa caracterização pudemos apontar algumas conclusões importantes brevemente discutidas como segue:

- o elemento caracterizado, denominado *hobo*^{v-a}, é um elemento *hobo* menor, de aproximadamente 1,2 kb de tamanho, que apresenta divergência de aproximadamente 18% em relação ao elemento canônico;
- este elemento é caracterizado como seqüência relacionada a *hobo* claramente defectiva como todas as outras, porém, mobilizável, pois apresenta as estruturas essenciais para mobilização bem conservadas;
- para sua mobilização, necessita de uma fonte de transposase ativa exógena que possa agir *in trans*. Dentre as possibilidades, sugerimos que o elemento canônico (autônomo) seja o candidato mais provável para desempenhar essa função, devido à similaridade de seqüência compartilhada entre esses elementos nas regiões críticas para mobilização, e pelas fortes evidências de invasão recente e atividade do

elemento canônico (Boussy e Daniels, 1991; Simmons, 1992; Loreto *et al.*, 1998c); Outras fontes de transposase, entretanto, não podem ser descartadas, visto que *hobo* pertence a uma grande superfamília de elementos relacionados, bem como mobilização cruzada já foi reportada entre membros dessa superfamília (Sundararajan *et al.*, 1999);

- *hobo^{v-a}* apresenta alta similaridade estrutural e de seqüência com o elemento *hobo* de *D. sechellia* (*hdelsech*) descrito por Periquet *et al.* (1994). Isto reforça a idéia de que *hdelsech* é também um elemento “relic” (provavelmente compartilhando um ancestral comum com *hobo^{v-a}* antes da especiação *D. simulans/D. sechellia*) e sugere um potencial de mobilização para esse elemento. A divergência nucleotídica estimada entre esses elementos é compatível com o tempo estimado de divergência das espécies. Se *hdelsech* é também mobilizável, podemos ter um exemplo de um transposon “relic” que se mantém mobilizável por relativamente longo tempo;
- seqüências similares a *hobo^{v-a}* estão ausentes no genoma de *D. melanogaster*. Se *hobo^{v-a}* e *hdelsech* são “relics” das primeiras invasões de *hobo* propostas por Boussy e Daniels (1991), essa ausência poderia ser explicada pela possível perda destas seqüências no ramo que deu origem a *D. melanogaster*;

- elementos *hobo*^{v-a} parecem constituir uma família particular de seqüências relacionadas, relativamente conservadas entre si e em diferentes populações de *D. simulans*;
- alguns “clusters” dentro dessa família podem ser distingüidos e os mesmos são compartilhados entre diferentes populações;
- apresentam polimorfismo de sítios de inserção e variabilidade no número de cópias, evidenciados por Southern Blot e ITAP, o que nos dá fortes indícios de atividade atual ou recente desses elementos no genoma dessas populações;
- compartilham muitas características estruturais e funcionais com elementos MITEs, o que nos leva a sugerir que elementos *hobo*^{v-a} podem ser ou uma nova família de MITEs de *Drosophila* ou, mais provavelmente, estariam se encaminhando para esse destino.

À parte das principais conclusões/discussões apresentadas aqui, os dados obtidos nessa tese nos instigam a fazer, ainda, algumas outras especulações a respeito da dinâmica das seqüências relacionadas a *hobo* descritas no presente estudo e suas possíveis interações com o elemento autônomo moderno no genoma de *D. simulans*. Se considerarmos, por exemplo, o ciclo de vida proposto de um transposon (Brookfield, 2005), podemos sugerir que *hobo*^{v-a} e o elemento canônico estariam passando por uma fase de competição entre elemento autônomo, que está em processo de invasão e aumento do número de cópias, *versus* elemento não-autônomo

que, mesmo sem agir como repressor direto do elemento autônomo compete por sua transposase *in trans* para sua mobilização e aumento do seu número de cópias. Esse elemento não autônomo (*hobo^{v-a}*), como já comentamos, teria se originado de invasões mais antigas de um outro elemento autônomo (que provavelmente se extinguiu) e agora interage com um elemento autônomo que invadiu recentemente o genoma.

Independente da origem dessas seqüências, outra questão a discutir é a do impacto desse tipo de seqüência na evolução do elemento autônomo. A proliferação de elementos não autônomos tem sido hipotetizada levar a extinção do elemento autônomo de origem através da competição pela transposase ativa produzida por aqueles elementos. Se, além dos elementos defectivos convencionais, outras formas de “competidores” pelas transposases ativas também estão presentes no genoma, seja como uma forma MITE ou mesmo mais branda (como “relics”), podemos esperar que o elemento autônomo sofra um impacto maior e se encaminhe cada vez mais para uma fase de eliminação do genoma, começando pela progressiva diminuição do número de cópias até sua provável extinção. Conseqüentemente, com o encerramento da produção de transposases ativas, elementos não autônomos (como os “relics” mobilizáveis) tendem a ser inativados e também perdidos, a menos que encontrem uma forma alternativa de sobrevivência, como mobilização cruzada, por exemplo.

Uma questão bastante intrigante, ainda, é a de como esses elementos não autônomos vêm se mantendo por tanto tempo, com um grau de conservação de seqüência que lhes permite ainda serem mobilizáveis e, provavelmente, ativos? Conforme discutido em Torres *et al.*, (2005), elementos *hobo*^{v-a} se manteriam mobilizáveis para escapar da eliminação, segundo a hipótese de Lerat *et al.* (2003) de um alto “turnover” de TEs no genoma. Mas como isto poderia acontecer? Mullins *et al.* (1989) sugerem que as ITRs e regiões flanqueadoras internas (ou subterminais) são as mais importantes para mobilização de elementos de Classe II (transposons). Então, para se manterem mobilizáveis, essas regiões devem se manter conservadas o suficiente para que alguma transposase relacionada possa reconhecê-las e, então, efetuar a mobilização do elemento. Nesse contexto, podemos propor, então, que possa haver seleção atuando, ao nível do elemento, para a conservação dessas regiões, o que garantiria sua mobilidade, atividade e, conseqüentemente, sua sobrevivência.

Silva e Kidwell (2000) têm proposto que a principal e talvez a única fonte de seleção realmente atuante sobre os TEs ocorra durante a transmissão horizontal, uma vez que esse seria o mecanismo essencial para a sua sobrevivência. No entanto, se considerarmos que a atividade (ou a mobilidade) é outra forma de sobrevivência de um elemento no genoma, podemos propor que a seleção também atue na conservação de regiões

críticas à mobilização (o outro mecanismo essencial para sobrevivência do elemento).

A continuidade de trabalhos com elementos *hobo*^{v-a} poderá contribuir para a compreensão de muitas das questões discutidas aqui e até mesmo outras que venham a ser levantadas. Assim, como perspectivas, sugerimos:

- aumento do número de “reads” para cada clone, pelo seqüenciamento dos mesmos, para montagem e análise da seqüência inteira dos elementos nas populações;
- expansão da amostragem populacional, com populações de diferentes procedências geográficas, para avaliar a conservação de *hobo*^{v-a} nessas populações;
- análise particular da região microsatélite interna desses elementos, para avaliar o grau de degeneração e/ou similaridade entre seqüências de diferentes populações;
- ensaio de mobilização de *hobo*^{v-a} para confirmar seu potencial de mobilidade;
- busca de *hobo*^{v-a} ou seqüências similares nos genomas disponíveis, especialmente de *D. simulans* e espécies relacionadas, para avaliação do número de cópias, estrutura e conservação dessas seqüências nessas espécies;
- caracterização de elementos *hobo*^{v-a} nas espécies crípticas de *D. simulans* (*D. mauritiana* e *D. sechellia*) como forma de avaliar a possível

origem, manutenção e relacionamento dessas seqüências entre as as espécies do complexo *melanogaster*.

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Anexo 1

Alinhamentos das seqüências dos elementos *hobo* canônico de *Drosophila melanogaster* (HFL1) e *hdelsech* de *Drosophila sechellia* com a seqüência do elemento *hobo*^{v-a} de *Drosophila simulans*.

Figura 5 – Alinhamento da seqüência do elemento *hobo* canônico de *Drosophila melanogaster* (HFL1) (GenBank M69216) com o elemento *hobo*^{v-a} de *Drosophila simulans* (GenBank AY764286).


```

* 1520 * 1540 * 1560 * 1580 * 1600 * 1620 * 1640 * 1660 * 1680 * 1700 * 1720 * 1740 * 1760 * 1780 * 1800 * 1820 * 1840 * 1860 * 1880
HFL1 : ATACTGGCGTAGTGGG TAAAAATG TTAGGTCAGCTGATATCCATGTAGATTTTAAATAATCATCTTTTAAAAGTTGTGGTAGATATCTTAGG : 1544
hoboVA : ----- TAAAAATG ----- : 479
          TAAAAATA
* 1620 * 1640 * 1660 * 1680 * 1700 * 1720 * 1740 * 1760 * 1780 * 1800 * 1820 * 1840 * 1860 * 1880
HFL1 : AGACTTGAAGCAATATTTAAGAAGTTGCAACATCTAGCTACCCATCTATATGCTTCGTAT TGCCATGCATCTCTTAAATTTTAGAATTTATGC : 1638
hoboVA : ----- TGCCCTTCATC ----- : 490
          TGCC T CATC
* 1700 * 1720 * 1740 * 1760 * 1780 * 1800 * 1820 * 1840 * 1860 * 1880
HFL1 : GAGCCGAATA TTTAGACGTTTCUGACAGCATTGCTTAAAGGAAAGAAATTTTGGAAATAATTCGTAAGATTTGGATGGCAAAATGTAAGCATAT : 1732
hoboVA : ----- : -
* 1800 * 1820 * 1840 * 1860 * 1880
HFL1 : GGGATAGGGGGCA TTTT TTTATATCCACCCGAGCACATCTTCAGGAAGAAGTATTCTTTGAAATAAAGGTGTTTTGCATTTTCACAAATTCAT : 1826
hoboVA : ----- TTTT TTTATAT ----- : 513
          TTTT TTTATAT
          TATT TT ATAA
          TT TT TTATAT
* 1900 * 1920 * 1940 * 1960 * 1980
HFL1 : AGTCCCAATTTTCATACACATTAAGCTTAGAATCTACAGAACTCCAGAACTCCAGAACTCCAGAACTCCAGAAAGTCTAGAAAGTCCCAATC : 1920
hoboVA : ----- : -
* 2000 * 2020 * 2040 * 2060
HFL1 : TTATTTCCAAAATAAAACAAAACAATATCTTCTGAAAACGAAATCTTCTCCCAAAGTTAGTAACTGAGTCTAAATTCACAACTTCAATGAATCTC : 2014
hoboVA : ----- TTAT ----- : 517
          TTAT
* 2080 * 2100 * 2120 * 2140 * 2160
HFL1 : CATTAGATGAATTTGAACCATATATTA GACAAAGAGTTCCATTTGTCARAAATTTTGAAGTAAATGAGTGGTGGAAATAAACGGAAAACCTTATA : 2108
hoboVA : ----- ATATATTA ----- : 525
          ATATATTA
          ATATATTA
* 2180 * 2200 * 2220 * 2240 * 2260
HFL1 : CCGTCAGTTGTCAAAGTTAGCATTTAAACTTTTATCAATACAGCCAGTAGCCAGCAGCTGAAAGAGTGTTCCTCCAGCAGGTAATATATA : 2202
hoboVA : ----- : -

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HFL1 : 2260 * 2280 * 2300 * 2320 * 2340 * 2296
hoboVA : ACAGAAAAGGAAATAGATTATGCCCAAATCTGTAGATAGCCCTCTTTTTCATTCCTATTACAAAACCTAACAACTGGCAATAGATAI : 2296
-----

HFL1 : 2360 * 2380 * 2400 * 2420 * 2440 * 2389
hoboVA : TCCCTCTTAAAGCTATATTTTATTTATTCCTTAAATTTTGTATTCCTTAACTTAACTATAAGTATTAAATTAATA- : 2389
-----TTAT-ATTATATTTTATAATATAATATAT--ATATAATAATAAATGTTTAAATTAATAATTTA-TAAATATA--TATTNATGTATAI : 607
TTAT A TATATTTTATA TAT AT T TAT TAAAT TTT TA A T T A TA TAA TATA TATT AT ATA

HFL1 : 2460 * 2480 * 2500 * 2520 * 2520 * 25
hoboVA : AATATAAGATTTGTTATTTGTTTACACATTTT-CATGCCAAATCCCTAAATAATGCAAGT-AATGAACTCCCTTATTTT--TAATAG--ATACTT : 2477
AATATAAATTAATTAATTA-ATGTTTATTCATTAATTTTCCCTTTTAAATTTTAAATGTAATTTTACGTAATAATAGTAATAGTAAATAG : 700
A TATAA AT TTA TGT A CAT A AT A T TAA AT T AA GT AAT A T ATA T TAATAG ATA T

40 * 2560 * 2580 * 2600 * 2620 * 2568
HFL1 : TTTA-AGCCGACTATGTTTATTATTTAGATTGAGACA-TTAAAAACCGTTAAATAT-CACCAAAATCCCGTCTTAAATTCCTTAACTTATGTT : 2568
hoboVA : CTAATAGACGATCTTATGACATTAATGTCCEAAATTTCAAAAAATCAATTTTATGTAAGAAATTTTGTGCAAT-AGGCTTTCAGCTAGCGA : 793
T A AG C A T T T T AT AT T A CA TT AAAAA G A AT AA AA GTC TT A G A T A T G

2640 * 2660 * 2680 * 2700 * 2720 * 2659
HFL1 : GTT-TGAAATGGAGCCCCCAITGAGTCCATCAAAAGAGCAAA--GACATGAGCACAAAATTTTCTTGGGTATTCCTTTTACCCCTTCATTTC : 2659
hoboVA : CTTCTGAAAATTACTTAGGCAATGATTCGAAACGGCTTGCACCCGACATGAGCACGAAAATTTTCTTGGGTATTCCTTTTACCCCTTCATTTC : 887
TT TGAAA AG A CA TGA TC A G G A GACATGAGCAC AAA TTTTCTTGGGTATTCCTTTTACCCCTTCATTTC

2740 * 2760 * 2780 * 2800 * 2820 * 2753
HFL1 : TTATACCCGTC CGCTTCCACCCATACAAATTTTAGGCGTACAAAAATGACCGAGAACTGCAGCCGCGATACA.AAAAAATGACCTGGGCGCGA : 2753
hoboVA : TTATACCCGTC CGCTTCCACCCATACAAATTTTAGGCGTAC-----AAAAATGACCTGGGCGCGA : 948
TTATACCCGTC CGCTTCCACCCATACAAATTTTAGGCGTAC

2840 * 2860 * 2880 * 2900 * 2847
HFL1 : TCGTTGACTGCTGCGTCCACTCACCATACGGCTCTTGGCGAGCAGGCTCGGGTGGTTTTTTTACTCGTAAAC.AAAACACAACGCTCGGTAAAC : 2847
hoboVA : TCGTTGACTGCTGCGTCCACTCACCATACGGCTCTTGGCGAGCAGGCTCGGGTGGTTTTTTTACTCGTAAAC-AAAACACAACGCTCGGTAAAC : 1041
TCGTTGACTGCTGCGTCCACTCACCATACGGCTCTTGGCGAGCAGGCTCGGGTGGTTTTTTTACTCGTAAAC AAAACACAACGCT CGGTAAAC

2920 * 2940 * 2960 * 2980 * 3000 * 2876
HFL1 : AGTCGACTTATTTTGTGTTTCCCGAAGT-----AG : 2876
hoboVA : ACCCGATATTTTGTGCGGAGTCTGCTGACCGCTTTTACTCGTACAAAACACAACGCTTGGTAAACACCCGATATTTTGTGCGCGAG : 1135
AC CGA TTTTGTG GC G A GT

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*      3020      *      3040      *      3060      *      3080      *
HFL1 : --GGTGTCAAAAAAACCAGGGGTGCCTAGAGTACCCGAGTGTGTTATCGGGTGGACGTAGAGTGCCAGTGGCGGGCTGCAGTTCTCTG : 2959
hoboVA : TAGGTGTCAAAAAACACGGGGTGCCTAGAGACCGAGTGTGTTATCGGGTGGACGTAGAGTGCCAGTGGCGGGCTGCAGTTCTCTG : 1220
GGTGTCAAAAAAACCAGGGGTGCCTAGAGACCGAGTGTGTTATCGGGTGGACGTAGAGTG C ACTGGCGGGCT CAGTTCTCTG

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Figura 6 - Alinhamentos das seqüências do elemento *hdelsech* de *Drosophila sechellia* com a seqüência do elemento *hobo*^{v-a} de *Drosophila simulans*.

```

*      20      *      40      *      60      *      80      *
hdelsech : CAGAGAACTGCAAGGGTGGCACATTTTATTACCACTCGACTCGACCCACAAATTTTGTGTG-----TGCTACCCGGCCAGCACATCG--CG : 83
hoboVA   : CAGAGAACTGCA--GGTGGATTITTA--GCCATC--GAT---CAACCAACAATTTTGTGTGCTGGTGTGTTACCCGGCCATGCACATCTGCTG : 84
          CAGAGAACTGCA GGTGG TTT CCA GA CA CC ACAATTTT GTGTG TGCTACCCGGCCA GCACATC G

          *      100      *      120      *      140      *      160      *      180
hdelsech : GGTACTTACAACACACACAGTATAATCTGACACAGAGACACAGCCAGACAAAGGATGAGTGCCTCTGGGTGCGGC : 174
hoboVA   : GGTACTTACAACACACACAGTATAATCTGACACAGTGTAGACAGACACCCCTGTTGTGCTGACCCGGAATCAATACGGTGCACCTGGGTGCGG : 176
          GGTACTTACAACACACAGTATA ATCTG ACA G AGACAAAGACACC C GTTGTGC C ACCCGAATCAATACGGTGC CTGGGTGCGG

          *      200      *      220      *      240      *      260      *
hdelsech : GGTGCGGATCAACAC : 217
hoboVA   : GTGCGCGATCAACTCTGGCACCCCATACGGGAGGTAGAGAGACAAACAGTAAAGCAGAAAAGAAAGCCCTAAAAGGGATGAGTGCACAAA-- : 267
          G GTGCGCGATCA AC C GCCTAAAAGGGATGAGTGCACAAA

          *      280      *      300      *      320      *      340      *      360
hdelsech : CACTTTGG---TTACCGT--AAACACATGGGTGTTTCCAAAAAATACTCGGGTGTTC-----AAAAATACTCGAGTGC : 288
hoboVA   : CACTTTGGGTTTACCGTAAACACATGGGTGTTTCCAAAAAATACTCGGGTGTTCAAAAATACTCGGGTGTAGTCTCTATCAGTCTATC : 359
          CACTT TGG TTACCGT AAACACATGGGTGTTTCCAAAAAATACTCGGGTGTTC AAAAATA TCG GTG T

          *      380      *      400      *      420      *      440      *      460
hdelsech : ----CTGTAGCTAGTGTGACTCAAGACAAGATGGTAACTCCATACGTTTACACTTCATACCTTTACACACTATTTCTTCGGCGTGGCTC : 376
hoboVA   : TACTGTGTAGCTAGTGTGACTCAGACAAGATGGTAACTCCATACGTTTACACTCATACGTTTACACACTATTTCTTCGGCGTGGCTC : 451
          CT GTAG TAGTGTGACTCA AGACAAGATGGTAACTCCATACGTTTACACT CATACGTTTACACACTATTTCTTCGGCGTGGCTC

          *      480      *      500      *      520      *      540      *
hdelsech : TACTTTTGTCAAAATACCTTGTATAAATATGCCCTTCATCTTATTTATATATTTATTTAATATATATATATATATATATATATAT : 461
hoboVA   : TACTTTTGTCAATACCTTGTATAAATATGCCCTTCATCTTATTTATATATTTATTTAATATATATATATATATATATATATATATAT : 542
          TA ACTTTTGTCA AATAC TTGTA AATATGCCCTTCATCTTAT T TATATTTATTTAATATATATATATATATATATATATAT TTAT

          *      560      *      580      *      600      *      620      *      640
hdelsech : ATAT-----TATATATAAATATAATAATAATAATAATAATAATAATAATAATAATAATAATAATAATAATAATAATAATAATAATA : 546
hoboVA   : ATATATAATAATAATAATAATAATAATAATAATAATAATAATAATAATAATAATAATAATAATAATAATAATAATAATAATAATAATA : 629
          ATAT TATATA TA TAAT T TATA ATA TATATAT TATAA TTA TATT A ATA AAT ATAA AT AATTAAT AT

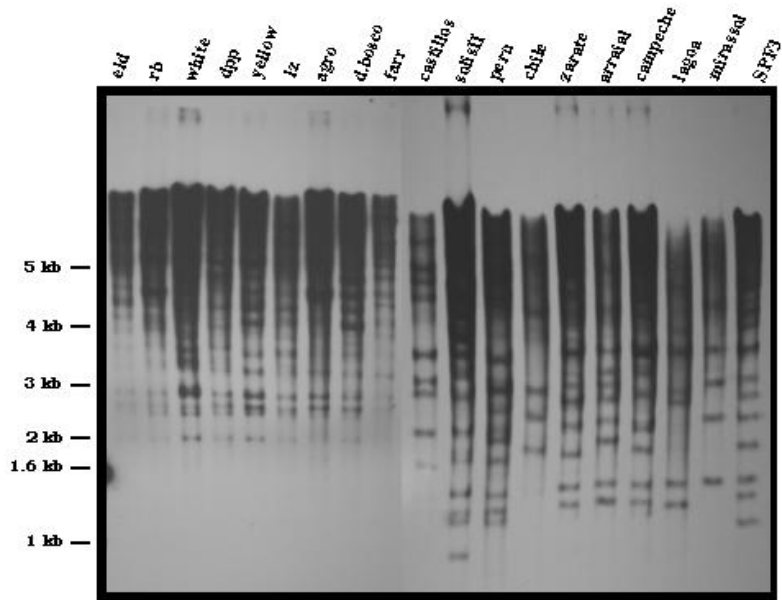
          *      660      *      680      *      700      *      720      *
hdelsech : AATATAAATATATAAATATAATAATAATAATAATAATAATAATAATAATAATAATAATAATAATAATAATAATAATAATAATAATA : 638
hoboVA   : AATATAAATATAAATATAATAATAATAATAATAATAATAATAATAATAATAATAATAATAATAATAATAATAATAATAATAATAATA : 718
          AAT ATAAT AT AA TT TT AAT ATTTAAAT TA ATTATAGTAAAAAATAAATAGT ATAGTAAATAGTCTAATAGACCGAAATCTATG

```


Anexo 2

Figuras de Southern blot, matrizes e alinhamentos referentes às seqüências descritas no Capítulo 3.

A)



B)

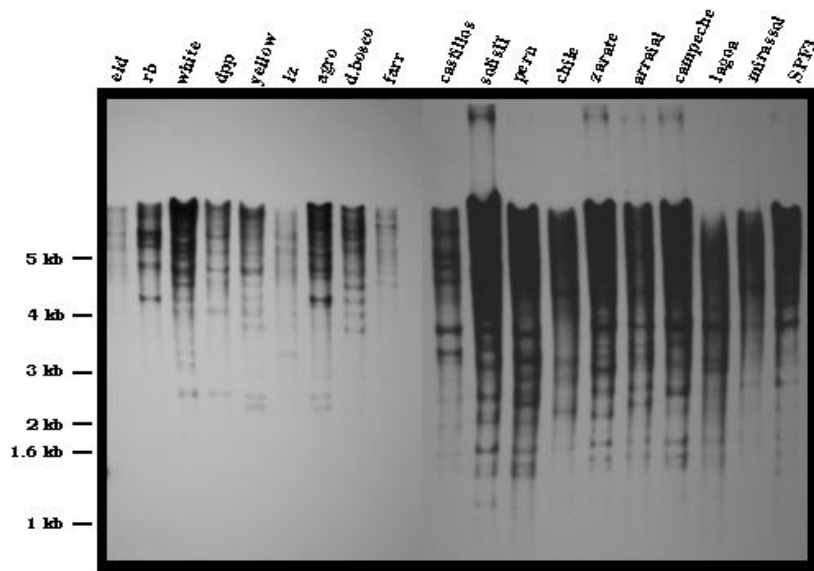


Figura 1- Southern blot do DNA genômico digerido com *Bgl*III de algumas linhagens de *Drosophila simulans* hibridizados com **A)** pHFL1 e **B)** pHVA. Os nomes das linhagens estão acima e o marcador de peso molecular está indicado à esquerda.

Figura 2 – Alinhamento das regiões subterminais 5' das seqüências *hobo*^{v-a} amplificadas com primers h^{v-a} 1s e h^{v-a} 2as, descritas no Capítulo 3. As siglas correspondem aos nomes das linhagens utilizadas e os números ao lado das siglas correspondem a clones diferentes de uma mesma linhagem.

```

MIR1 : CATAACGGAGGGGTAGAGAAACAAAACAGCTCAGAAAAGACAAATGCCCTAAAAGGATGAGTACAAAAACACTTTGTGG-      80      *
YEL2 : CATAACGGAGGGGTAGAGAAACAAAACAGCTCAGAAAAGACAAATGCCCTAAAAGGATGAGTACAAAAACACTTTGTGG-      80      *
CHI3 : CATAACGGAGGGGTAGAGAAACAAAACAGCTCAGAAAAGACAAATGCCCTAAAAGGATGAGTACAAAAACACTTTGTGG-      80      *
YEL1 : CATAACGGAGGGGTAGAGAAACAAAACAGCTCAGAAAAGACAAATGCCCTAAAAGGATGAGTACAAAAACACTTTGTGG-      80      *
MIR5 : CATAACGGAGGGGTAGAGAAACAAAACAGCTCAGAAAAGACAAATGCCCTAAAAGGATGAGTACAAAAACACTTTGTGG-      80      *
MIR4 : CATAACGGAGGGGTAGAGAAACAAAACAGCTCAGAAAAGACAAATGCCCTAAAAGGATGAGTACAAAAACACTTTGTGG-      80      *
GOI2 : CATAACGGAGGGGTAGAGAAACAAAACAGCTCAGAAAAGACAAATGCCCTAAAAGGATGAGTACAAAAACACTTTGTGG-      80      *
GOI1 : CATAACGGAGGGGTAGAGAAACAAAACAGCTCAGAAAAGACAAATGCCCTAAAAGGATGAGTACAAAAACACTTTGTGG-      80      *
CHI2 : CATAACGGAGGGGTAGAGAAACAAAACAGCTCAGAAAAGACAAATGCCCTAAAAGGATGAGTACAAAAACACTTTGTGG-      80      *
MIR2 : CATAACGGAGGGGTAGAGAAACAAAACAGCTCAGAAAAGACAAATGCCCTAAAAGGATGAGTACAAAAACACTTTGTGG-      80      *
DB1 : CATAACGGAGGGGTAGAGAAACAAAACAGCTCAGAAAAGACAAATGCCCTAAAAGGATGAGTACAAAAACACTTTGTGG-      80      *
CATAACGGAGGGGTAGAGAAACAAAACAGCTCAGAAAAGACAAATGCCCTAAAAGGATGAGTACAAAAACACTTTGTGG      80      *

MIR1 : ATGGGTGTTTCCAAAAATACTCGGAGTGGTTTCGAAAAATACTCGGGTAAATAGT-CTCTATCAGTCTATCTAC--TCCTG-TAGATAGTCC--AGTCAC : 186
YEL2 : ATGGGTGTTTCCAAAAATACTCGGAGTGGTTTCGAAAAATACTCGGGTAAATAGT-CTCTATCAGTCTATCTAC--TCCTG-TAGATAGTCC--AGTCAC : 187
CHI3 : ATGGGTGTTTCCAAAAATACTCGGGA-----ATATTT-CTCTATCAGTCTATCTAC--TCCTG-TAGATAGTCC--AGTCAC : 166
YEL1 : ATGGGTGTTTCCAAAAATACTCGGTTTTCGAAAAATACTCGGGTAAATAGT-CTCTATCAGTCTATCTAC--TCCTG-TAGATAGTCC--AGTCAC : 186
MIR5 : ATGGGTGTTTCCAAAAATACTCGGTTTTCGAAAAATACTCGGGTAAATAGT-CTCTATCAGTCTATCTAC--TCCTG-TAGATAGTCC--AGTCAC : 186
MIR4 : ATGGGTGTTTCCAAAAATACTCGGTTTTCGAAAAATACTCGGGTAAATAGT-CTCTATCAGTCTATCTAC--TCCTG-TAGATAGTCC--AGTCAC : 186
GOI2 : ATGGGTGTTTCCAAAAATACTCGGTTTTCGAAAAATACTCGGGTAAATAGT-CTCTATCAGTCTATCTAC--TCCTG-TAGATAGTCC--AGTCAC : 186
GOI1 : ATGGGTGTTTCCAAAAATACTCGGTTTTCGAAAAATACTCGGGTAAATAGT-CTCTATCAGTCTATCTAC--TCCTG-TAGATAGTCC--AGTCAC : 188
CHI2 : ATGGGTGTTTCCAAAAATACTCGGTTTTCGAAAAATACTCGGGTAAATAGT-CTCTATCAGTCTATCTAC--TCCTG-TAGATAGTCC--AGTCAC : 188
MIR2 : ATGGGTGTTTCCAAAAATACTCGGTTTTCGAAAAATACTCGGGTAAATAGT-CTCTATCAGTCTATCTAC--TCCTG-TAGATAGTCC--AGTCAC : 191
DB1 : ATGGGTGTTTCCAAAAATACTCGGTTTTCGAAAAATACTCGGGTAAATAGT-CTCTATCAGTCTATCTAC--TCCTG-TAGATAGTCC--AGTCAC : 193
ATGGGTGTTTCCAAAAATACTCGGTTgttc aaasatactgggtt AT T TcTATcagTcTATctAc TcTtg TAGATA tcg AgtcAc

```


Figura 3 – Alinhamento das regiões subterminais 3' das seqüências *hobo*^{v-a} amplificadas com primers h^{v-a} 1s e h^{v-a} 2as, descritas no Capítulo 3. As siglas correspondem aos nomes das linhagens utilizadas e os números ao lado das siglas correspondem a clones diferentes de uma mesma linhagem.


```

DPP6 : CACCAGTTTCGAAATCAATTGGCCCTTACTAAATTTTTTCACAAGTCGGCTAGCTGAATACTCTTAATGACAAATAAATTCCTTAC- : 367
YEL2 : CACTCGTTTCGAAATCAATTGGCCCTTACTAAATTTTTTCACAAGTCGGCTAGCTGAATACTCTTAATGACAAATAATTCCTTAC- : 366
DPP3 : CACCAGTTTCGAAATCAATTGGCCCTTACTAAATTTTTTCACAAGTCGGCTAGCTGAATACTCTTAATGACAAATAATTCCTTAC- : 367
DB1 : CACCAGTTTCGAGTCAATTGGCCCTTACTAAATTTTTTCACAAGTCGGCTAGCTGAATACTCTTAATGACAAATAATTCCTTGC- : 366
DPP4 : CACCAGTTTCAAATTCATTGGCCCTTACTAAATTTTTTCACAAGTCGGCTAGCTGAATACTCTTAATGACAAATAATTCCTTGC- : 366
GOI1 : CACCAGTTTCGAAATCAATTGGCCCTTACTAAATTTTTTCACAAGTCGGCTAGCTGAATACTCTTAATGACAAATAATTCCTTGC- : 366
DPP2 : CACCAGTTTCGAAATCAATTGGCCCTTACTAAATTTTTTCACAAGTCGGCTAGCTGAATACTCTTAATGACAAATAATTCCTTGC- : 366
GOI2 : CACCAGTTTCGAAATCAATTGGCCCTTACTAAATTTTTTCACAAGTCGGCTAGCTGAATACTCTTAATGACAAATAATTCCTTGC- : 366
CHI2 : CACCAGTTTCGAAATCAATTGGCCCTTACTAAATTTTTTCACAAGTCGGCTAGCTGAATACTCTTAATGACAAATAATTCCTTGC- : 366
YEL1 : CACCAGTTTCGAAATCAATTGGCCCTTACTAAATTTTTTCACAAGTCGGCTAGCTGAATACTCTTAATGACAAATAATTCCTTGC- : 366
CACCCGTTTCGAAATCAATTGGCCCTTACTAAATTTTTTCACAAGTCGGCTAGCTGAATACTCTTAATGACAAATAATTCCTT C

```

Figura 4 – Montagem dos alinhamentos das regiões subterminais 5' e 3' das seqüências *hobo*^{v-a} amplificadas com primers *h*^{v-a} 1s e *h*^{v-a} 2as, descritas no Capítulo 3 juntamente com as seqüências dos elementos *hobo*^{v-a} (AY764286) e *hdelsech* (X77577) nas regiões correspondentes. As siglas correspondem aos nomes das linhagens utilizadas e os números ao lado das siglas correspondem a clones diferentes de uma mesma linhagem.

```

MIR2      *      *      *      *      *      *      *      *
DB1       -----
CHI2      -----
CHI3      -----
DB 2      -----
MIR5      -----
MIR4      -----
MIR1      -----
GOI2      -----
GOI1      -----
YEL1      -----
hoboVA    CACAGAACTGAAGGTGGATTTTGGCCATCGATCAACC AACAAATTTTGGTGTGCTGGTGTGCTACCCGGCCATGGACACTGTGCTGGGTA CTTACAA
HVAw      -----
YEL2      -----
hdelsech -----
DPP4      -----
DPP2      -----
DPP3      -----
DPP6      -----

```

95

```

MIR2      *      *      *      *      *      *      *      *
DB1       -----
CHI2      -----
CHI3      -----
DB 2      -----
MIR5      -----
MIR4      -----
MIR1      -----
GOI2      -----
GOI1      -----
YEL1      -----
hoboVA    ACACACAATAATATCTGCACATGTAGACAAGACACCCCTGTTGTGCCCTGACCCGAATCAATACGGGTGACCTGGGGTCCGGGTGTCGCCGATCATA
HVAw      -----
YEL2      -----
hdelsech -----
DPP4      -----
DPP2      -----
DPP3      -----
DPP6      -----

```

190

```

200          *          220          *          240          *          260          *          280
MIR2 : ---CATAACGGAGCGGTAGACAAGA CA AACAGTCAGAAAAA GACAAA TGCCCTAAA AAGGGA TGA GT CACAAA AGCACTTGTG-GGTTTAC : 85
DB1 : ---CATAACGGAGCGGTAGACAAGA CA AACAGTCAGAAAAA GACAAA TGCCCTAAA AAGGGA TGA GT CACAAA AGCACTTGTGTTGTGTTAC : 86
CHI2 : ---CATAACGGAGCGGTAGACAAGA CA AACAGTCAGAAAAA GACAAA TGCCCTAAA AAGGGA TGA GT CACAAA AGCACTTGTG-GGTTTAC : 85
CHI3 : ---CATAACGGAGCGGTAGACAAGA CA AACAGTCAGAAAAA GACAAA TGCCCTAAA AAGGGA TGA GT CACAAA AGCACTTGTGG-ETTTAC : 85
DB2 : ---CATAACGGAGCGGTAGACAAGA CA AACAGTCAGAAAAA GACAAA TGCCCTAAA AAGGGA TGA GT CACAAA AGCACTTGTGG-ETTTAC : 85
MIR5 : ---CATAACGGAGCGGTAGACAAGA CA AACAGTCAGAAAAA GACAAA TGCCCTAAA AAGGGA TGA GT CACAAA AGCACTTGTGG-ETTTAC : 85
MIR4 : ---CATAACGGAGCGGTAGACAAGA CA AACAGTCAGAAAAA GACAAA TGCCCTAAA AAGGGA TGA GT CACAAA AGCACTTGTGG-ETTTAC : 85
MIR1 : ---CATAACGGAGCGGTAGACAAGA CA AACAGTCAGAAAAA GACAAA TGCCCTAAA AAGGGA TGA GT CACAAA AGCACTTGTGG-ETTTAC : 85
GOI2 : ---CATAACGGAGCGGTAGACAAGA CA AACAGTCAGAAAAA GACAAA TGCCCTAAA AAGGGA TGA GT CACAAA AGCACTTGTGG-ETTTAC : 85
GOI1 : ---CATAACGGAGCGGTAGACAAGA CA AACAGTCAGAAAAA GACAAA TGCCCTAAA AAGGGA TGA GT CACAAA AGCACTTGTAGTGTTTAC : 86
YEL1 : ---CATAACGGAGCGGTAGACAAGA CA AACAGTCAGAAAAA GACAAA TGCCCTAAA AAGGGA TGA GT CACAAA AGCACTTGTGG-ETTTAC : 85
hoboVA : CTCGGCACCCATAACGGAGCGGTAGACAAGA CA AACAGT-AAAGCAGAAA GAA CCGCTAAA AAGGGA TGA GT CACAAA -CACTTGTGG-ETTTAC : 282
HVAw : ---CATAACGGAGCGGTAGACAAGA CA AACAGT-AAAGCAGAAA GAA CCGCTAAA AAGGGA TGA GT CACAAA -CACTTGTGG-ETTTAC : 83
YEL2 : ---CATAACGGAGCGGTAGACAAGA CA AACAGTCAGAAAAA GACAAA TGCCCTAAA AAGGGA TGA GT CACAAA AGCACTTGTGG-ETTTAC : 85
hdels1ech : -----TCCCTAAA AAGGGA TGA GT CACAAA AGCACTTATGGA TGG-----TTTAC : 40
DPP4 : -NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN : 94
DPP2 : -NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN : 94
DPP3 : -NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN : 94
DPP6 : -----NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN : 14
          qcctaaaaagqatgagt acaaaa cactt t          ttac

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          *          300          *          320          *          340          *          360          *          380
MIR2 : C-GTTAAACACATGGGTGTTCCAAAAATACCTGGGTGTTTCGAAAAATACTCGGGTAAU-----TATATTA---TATTTATTATAACGTTTTTA : 172
DB1 : CTGTTAAACACATGGGTGTTCCAAAAATACCTGGGTGTTTCGAAAAATACTCGGGTAAU-----TATATTA---TATTTATTATAACGTTTTTA : 174
CHI2 : CGTATAACACATGGGTGTTCCAAAAATACCTGGGTGTTTCGAAAAATACTCGGGTAAUAGTCTCTATCACTCTACTCTCTGGTAGATAT : 180
CHI3 : C-GTTAAACACATGGGTGTTCCAAAAATACCTGGGTGTTTCGAAAAATACTCGGGT-----AAATTTCTCTATCACTCTATCTA CTCCTGTA GATAGT : 158
DB2 : CTGTTAAACACATGGGTGTTCCAAAAATACCTGGGTGTTTCGAAAAATACTCGGGTAAUAGTCTCTATCACTCTACTCTCTGTA GATAGT : 179
MIR5 : C-GTTAAACACATGGGTGTTCCAAAAATACCTGGGTGTTTCGAAAAATACTCGGGTAAUAGTCTCTATCACTCTATCTA CTCCTGTA GATAGT : 178
MIR4 : T-GTTAAACACATGGGTGTTCCAAAAATACCTGGGTGTTTCGAAAAATACTCGGGTAAUAGTTTCTATCACTCTACTCTCTGTA GATAGT : 178
MIR1 : C-GTTAAACACATGGGTGTTCCAAAAATACCTGGGTGTTTCGAAAAATACTCGGGTAAUAGTCTCTATCACTCTATCTA CTCCTGTA GATAGT : 178
GOI2 : C-GTTAAACACATGGGTGTTCCAAAAATACCTGGGTGTTTCGAAAAATACTCGGGTAAUAGTCTCTATCACTCTACTCTCTGTA GATAGT : 178
GOI1 : CTGTTAAACACATGGGTGTTCCAAAAATACCTGGGTGTTTCGAAAAATACTCGGGTAAUAGTCTCTATCACTCTATCTA CTCCTGTA GATAGT : 180
YEL1 : C-GTTAAACACATGGGTGTTCCAAAAATACCTGGGTGTTTCGAAAAATACTCGGGTAAUAGTCTCTATCACTCTACTCTCTGTA GATAGT : 178
hoboVA : C-GTTAAACACATGGGTGTTCCAAAAATACCTGGGTGTTTCGAAAAATACTCGGGTAAUAGTCTCTATCACTCTACTCTCTGTA GATAGT : 375
HVAw : C-GTTAAACACATGGGTGTTCCAAAAATACCTGGGTGTTTCGAAAAATACTCGGGTAAUAGTCTCTATCACTCTACTCTCTGTA GATAGT : 176
YEL2 : CTGTTAAACACATGGGTGTTCCAAAAATACCTGGGTGTTTCGAAAAATACTCGGGTAAUAGTCTCTATCACTCTACTCTCTGTA GATAGT : 179
hdels1ech : C--GTAAACACATGGGTGTTCCAAAAATACCTGGGTGTTTCGAAAAATACTCGA GTGG-----CCTGTA GGTAGT : 110
DPP4 : NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN : 189
DPP2 : NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN : 189
DPP3 : NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN : 189
DPP6 : NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN : 109
          taacacacgggtggtctccaaaaatactcgg gt          t          t

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MIR2 : TTATTTAAATCTTAATATAGTAAAA--ATAAATAGTAAATAGTAAATAACCTAAAGGCTGAAATCTAATGTACATAAA--TGTCACGAAA : 254
DB1 : TTATTTAAATGTAAATATAGTAAAA--ATAAATAGTAAATAGTAAATAACCTAAAGGCTGAAATCTAATGTACATAAA--TGTCACAAA : 256
CHI2 : CGAGTCA CAGACAGA GC -GTAA CTCCTACGTTTACAC TCCATAGCTTACACTATTCCTCCGGCGTGGCTCTAGACTTTGTCGGAATC : 273
CHI3 : CGAGTCA CAGACAGA GC -GTAA CTCCTACGTTTACAC TCCATAGCTTACACTATTCCTCCGGCGTGGCTCTAGACTTTGTCGGAATC : 251
DB2 : CGAGTCA CAGACAGA GC -GTAA CTCCTACGTTTACAC TCCATAGCTTACACTATTCCTCCGGCGTGGCTCTAGACTTTGTCGGAATC : 272
MIR5 : CGAGTCA CAGACAGA GC -GTAA CTCCTACGTTTACAC TCCATAGCTTACACTATTCCTCCGGCGTGGCTCTAGACTTTGTCGGAATC : 271
MIR4 : CGAGTCA CAGACAGA GC -GTAA CTCCTACGTTTACAC TCCATAGCTTACACTATTCCTCCGGCGTGGCTCTAGACTTTGTCGGAATC : 272
MIR1 : CGAGTCA CAGACAGA GC -GTAA CTCCTACGTTTACAC TCCATAGCTTACACTATTCCTCCGGCGTGGCTCTAGACTTTGTCGGAATC : 271
GOI2 : CGAGTCA CAGACAGA GC -GTAA CTCCTACGTTTACAC TCCATAGCTTACACTATTCCTCCGGCGTGGCTCTAGACTTTGTCGGAATC : 271
GOI1 : CGAGTCA CAGACAGA GC -GTAA CTCCTACGTTTACAC TCCATAGCTTACACTATTCCTCCGGCGTGGCTCTAGACTTTGTCGGAATC : 273
YEL1 : CGAGTCA CAGACAGA GC -GTAA CTCCTACGTTTACAC TCCATAGCTTACACTATTCCTCCGGCGTGGCTCTAGACTTTGTCGGAATC : 271
hoboVA : CGAGTCA CAGACAGA GC -GTAA CTCCTACGTTTACAC TCCATAGCTTACACTATTCCTCCGGCGTGGCTCTAGACTTTGTCGGAATC : 468
HVAw : CGAGTCA CAGACAGA GC -GTAA CTCCTACGTTTACAC TCCATAGCTTACACTATTCCTCCGGCGTGGCTCTAGACTTTGTCGGAATC : 269
YEL2 : CGAGTCA CAGACAGA GC -GTAA CTCCTACGTTTACAC TCCATAGCTTACACTATTCCTCCGGCGTGGCTCTAGACTTTGTCGGAATC : 272
hdels1ech : CGAGTCA CAGACAGA GC -GTAA CTCCTACGTTTACAC TCCATAGCTTACACTATTCCTCCGGCGTGGCTCTAGACTTTGTCGGAATC : 203
DPP4 : MNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN : 284
DPP2 : MNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN : 284
DPP3 : MNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN : 284
DPP6 : MNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN : 204

a a a a t qtaa ata t a a t a q t a a a t c t c t t a t c t a
480 * 500 * 520 * 540 * 560 *
T-----TCATTCAAAATCAA--TTATG-TAGGAAATATTCGCATACAGTATCAGCTACCGACTGTGA AAAATTA GT----- : 328
DB1 : T-----TCATTCAAAATCAA--TTATG-CAA GAAATATTTGCATACAGTATCAGCTACCGACTGTGA AAAATTA GTAAAGT----- : 334
CHI2 : TAACCTTTTGMNMTTCAAAATCAAATTA TGGCAAGGATTTGCATACAGTATCAGCTACCGACTGTGA AAAATTA GTAAAGT----- : 362
CHI3 : T-----TGTAAAATATGCCCTTCATCTTATTATAT--ATT-ITATTAAATATAAT-ATTAAATATAATTTTA----- : 319
DB2 : T-----TGTAAAATATGCCCTTCATCTTATTATAT--ATT-ITATTAAATATAAT-ATTAAATATAATTTTA----- : 340
MIR5 : T-----TGTAAAATATGCCCTTCATCTTATTATAT--ATT-ITATTAAATATAAT-ATTAAATATAATTTTA----- : 339
MIR4 : T-----TGTAAAATATGCCCTTCATCTTATTATAT--ATT-ITATTAAATATAAT-ATTAAATATAATTTTA----- : 340
MIR1 : T-----TGTAAAATATGCCCTTCATCTTATTATAT--ATA-ITATTAAATATAAT-ATTAAATATAATTTTA----- : 337
GOI2 : T-----TGTAAAATATGCCCTTCATCTTATTATAT--ATA-ITATTAAATATAAT-ATTAAATATAATTTTA----- : 339
GOI1 : T-----TGTAAAATATGCCCTTCATCTTATTATAT--ATT-ITATTAAATATAAT-ATTAAATATAATTTTA----- : 341
YEL1 : T-----TGTAAAATATGCCCTTCATCTTATTATAT--ATT-ITATTAAATATAAT-ATTAAATATAATTTTA----- : 339
hoboVA : T-----TGTAAAATATGCCCTTCATCTTATTATAT--ATTAAATATAATATAATTTTAATTTTAACTTTTATTACT : 549
HVAw : T-----TGTAAAATATGCCCTTCATCTTATTATAT--ATTAAATATAATATAATTTTAAATTTTAACTTTTAACT : 340
YEL2 : AIGAAAATATGCCCTTCATCT--TCATAT--ATA-ITATTAAATATAAT-ATTAAATATAATTTTA----- : 338
hdels1ech : TGTAAATATGCCCTTCATCTTATTAT--TATATATAT--TAAATATAAT-ATTAAATATAATTTTA----- : 269
DPP4 : M-----MNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN : 356
DPP2 : M-----MNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN : 356
DPP3 : M-----MNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN : 356
DPP6 : M-----MNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN : 276

t t aa atg tt at tat t tt t a t a t t a a t t a a t t a

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	960	*	980	*	1000	*	1020	*	1040	
MIR2	----		----		----		----		----	-
DB1	TTTCTTGGGTATCCCTTTTACCITCAITTCITATACCGTGCCTTCCACCCATACAAAATTTAGGGGTACAAAAATGACC									614
CHI2	TTTCTTGGGTATCCCTTTTACCITCAITTCITATACCGTGCCTTCCACCCATACAAAATTTAGGGGTACAAAAATGACC									489
CHI3	-----									-
DB2	-----									-
MIR5	-----									-
MIR4	-----									-
MIR1	-----									-
GOI2	TTTCTTGGGTATCCCTTTTACCITCAITTCITATACCGTGCCTTCCACCCATACAAAATTTAGGGGTACAAAAATGACC									546
GOI1	TTTCTTGGGTATCCCTTTTACCITCAITTCITATACCGTGCCTTCCACCCATACAAAATTTAGGGGTACAAAAATGACC									613
YEL1	TTTCTTGGGTATCCCTTTTACCITCAITTCITATACCGTGCCTTCCACCCATACAAAATTTAGGGGTACAAAAATGACC									546
hoboVA	TTTCTTGGGTATCCCTTTTACCITCAITTCITATACCGTGCCTTCCACCCATACAAAATTTAGGGGTACAAAAATGACC									1011
HVAw	TTTCTTGGGTATCCCTTTTACCITCAITTCITATACCGTGCCTTCCACCCATACAAAATTTAGGGGTACAAAAATGACC									609
YEL2	TTTCTTGGGTATCCCTTTTACCITCAITTCITATACCGTGCCTTCCACCCATACAAAATTTAGGGGTACAAAAATGACC									609
hdelsech	TTTCTTGGGTATCCCTTTTACCITCAITTCITATACCGTGCCTTCCACCCATACAAAATTTAGGGGTACAAAAATGACC									550
DPP4	TTTCTTGGGTATCCCTTTTACCITCAITTCITATACCGTGCCTTCCACCCATACAAAATTTAGGGGTACAAAAATGACC									628
DPP2	TTTCTTGGGTATCCCTTTTACCITCAITTCITATACCGTGCCTTCCACCCATACAAAATTTAGGGGTACAAAAATGACC									628
DPP3	TTTCTTGGGTATCCCTTTTACCITCAITTCITATACCGTGCCTTCCACCCATACAAAATTTAGGGGTACAAAAATGACC									629
DPP6	TTTCTTGGGTATCCCTTTTACCITCAITTCITATACCGTGCCTTCCACCCATACAAAATTTAGGGGTACAAAAATGACC									549
MIR2	-----									-
DB1	TTGCGCGGATCGTTGACTGTGCCTCCACTCACCCATACGGCTCTTGCACAGCAGGCTCGGGTGGTTTTTTA									687
CHI2	TTGCGCGGATCGTTGACTGTGCCTCCACTCACCCATACGGCTCTTGCACAGCAGGCTCGGGTGGTTTTTTA									562
CHI3	-----									-
DB2	-----									-
MIR5	-----									-
MIR4	-----									-
MIR1	-----									-
GOI2	TTGCGCGGATCGTTGACTGTGCCTCCACTCACCCATACGGCTCTTGCACAGCAGGCTCGGGTGGTTTTTTA									619
GOI1	TTGCGCGGATCGTTGACTGTGCCTCCACTCACCCATACGGCTCTTGCACAGCAGGCTCGGGTGGTTTTTTA									686
YEL1	TTGCGCGGATCGTTGACTGTGCCTCCACTCACCCATACGGCTCTTGCACAGCAGGCTCGGGTGGTTTTTTA									619
hoboVA	TTGCGCGGATCGTTGACTGTGCCTCCACTCACCCATACGGCTCTTGCACAGCAGGCTCGGGTGGTTTTTTA									1084
HVAw	TTGCGCGGATCGTTGACTGTGCCTCCACTCACCCATACGGCTCTTGCACAGCAGGCTCGGGTGGTTTTTTA									682
YEL2	TTGCGCGGATCGTTGACTGTGCCTCCACTCACCCATACGGCTCTTGCACAGCAGGCTCGGGTGGTTTTTTA									682
hdelsech	AGCCCCATACAAAATGACTGTGCCTCCACTCACCCATACGGCTCTTGCACAGCAGGCTCGGGTGGTTTTTTA									645
DPP4	TTGCGCGGATCGTTGACTGTGCCTCCACTCACCCATACGGCTCTTGCACAGCAGGCTCGGGTGGTTTTTTA									701
DPP2	TTGCGCGGATCGTTGACTGTGCCTCCACTCACCCATACGGCTCTTGCACAGCAGGCTCGGGTGGTTTTTTA									701
DPP3	TTGCGCGGATCGTTGACTGTGCCTCCACTCACCCATACGGCTCTTGCACAGCAGGCTCGGGTGGTTTTTTA									702
DPP6	TTGCGCGGATCGTTGACTGTGCCTCCACTCACCCATACGGCTCTTGCACAGCAGGCTCGGGTGGTTTTTTA									622

	*	1160	*	1180	*	1200	*	1220	*										
MIR2	----																		
DB1	CTCGTAA	CAAAA	CACAAC	GTGGTAA	AAACAC	CCGGAAT	ATTTTC	TGTTGCCG	CAAGTAGG	GTGTCA	AAAAAA	GAC	GGGGTG	CC	TAGAG-	ACC	GAGT	781	
CHI2	CTCGTAA	CAAAA	CACAAC	GTGGTAA	AAACAC	CCGGAAT	ATTTTC	TGTTGCCG	CAAGTAGG	GTGTCA	AAAAAA	GAC	GGGGTG	CC	TAGAG-	ACC	GAGT	656	
CHI3	-----																		
DB2	-----																		
MIR5	-----																		
MIR4	-----																		
MIR1	-----																		
GOI2	CTCGTAA	CAAAA	CACAAC	GTGGTAA	AAACAC	CCGGAAT	ATTTTC	TGTTGCCG	CAAGTAGG	GTGTCA	AAAAAA	GAC	GGGGTG	CC	TAGAG-	ACC	GAGT	713	
GOI1	CTCGTAA	CAAAA	CACAAC	GTGGTAA	AAACAC	CCGGAAT	ATTTTC	TGTTGCCG	CAAGTAGG	GTGTCA	AAAAAA	GAC	GGGGTG	CC	TAGAG-	ACC	GAGT	780	
YE11	CTCGTAA	CAAAA	CACAAC	GTGGTAA	AAACAC	CCGGAAT	ATTTTC	TGTTGCCG	CAAGTAGG	GTGTCA	AAAAAA	GAC	GGGGTG	CC	TAGAG-	ACC	GAGT	713	
hoboVA	CTCGTAA	CAAAA	CACAAC	GTGGTAA	AAACAC	CCGGAAT	ATTTTC	TGTTGCCG	CAAGTAGG	GTGTCA	AAAAAA	GAC	GGGGTG	CC	TAGAG-	ACC	GAGT	1172	
HVAw	CTCGTAA	CAAAA	CACAAC	GTGGTAA	AAACAC	CCGGAAT	ATTTTC	TGTTGCCG	CAAGTAGG	GTGTCA	AAAAAA	GAC	GGGGTG	CC	TAGAG-	ACC	GAGT	770	
YE12	CTCGTAA	CAAAA	CACAAC	GTGGTAA	AAACAC	CCGGAAT	ATTTTC	TGTTGCCG	CAAGTAGG	GTGTCA	AAAAAA	GAC	GGGGTG	CC	TAGAG-	ACC	GAGT	776	
hd1sech	CTCGTAA	CAAAA	CACAAC	GTGGTAA	AAACAC	CCGGAAT	ATTTTC	TGTTGCCG	CAAGTAGG	GTGTCA	AAAAAA	GAC	GGGGTG	CC	TAGAG-	ACC	GAGT	740	
DPP4	CTCGTAA	CAAAA	CACAAC	GTGGTAA	AAACAC	CCGGAAT	ATTTTC	TGTTGCCG	CAAGTAGG	GTGTCA	AAAAAA	GAC	GGGGTG	CC	TAGAG-	ACC	GAGT	795	
DPP2	CTCGTAA	CAAAA	CACAAC	GTGGTAA	AAACAC	CCGGAAT	ATTTTC	TGTTGCCG	CAAGTAGG	GTGTCA	AAAAAA	GAC	GGGGTG	CC	TAGAG-	ACC	GAGT	795	
DPP3	CTCGTAA	CAAAA	CACAAC	GTGGTAA	AAACAC	CCGGAAT	ATTTTC	TGTTGCCG	CAAGTAGG	GTGTCA	AAAAAA	GAC	GGGGTG	CC	TAGAG-	ACC	GAGT	796	
DPP6	CTCGTAA	CAAAA	CACAAC	GTGGTAA	AAACAC	CCGGAAT	ATTTTC	TGTTGCCG	CAAGTAGG	GTGTCA	AAAAAA	GAC	GGGGTG	CC	TAGAG-	ACC	GAGT	716	
MIR2	1240	*	1260	*	1280														
DB1	-----																		
CHI2	GTTTATC	GGGTGG	ACG	-----															797
CHI3	GTTTATC	GGGTGG	ACG	-----															672
DB2	-----																		
MIR5	-----																		
MIR4	-----																		
MIR1	-----																		
GOI2	GTTTATC	GGGTGG	ACG	-----															729
GOI1	GTTTATC	GGGTGG	ACG	-----															796
YE11	GTTTATC	GGGTGG	ACG	-----															729
hoboVA	GTTTATC	GGGTGG	ACG	TAGAG	TGC	CCAG	TTC	CTC	TG	1220									
HVAw	GTTTATC	GGGTGG	AC	-----															785
YE12	GTTTATC	GGGTGG	ACG	-----															792
hd1sech	GTTTATC	GGGTGG	ACG	-----															756
DPP4	GTTTATC	GGGTGG	ACG	-----															811
DPP2	GTTTATC	GGGTGG	ACG	-----															811
DPP3	GTTTATC	GGGTGG	ACG	-----															812
DPP6	GTTTATC	GGGTGG	ACG	-----															732

CN x CN	RB	DPP	WH	LZ	YEL	ELD	PER	ZAR	SOL1	MIR	ARR	CAMP	LINE2	LAG	CAST	AGRO	FARR	CHI
RB	*	33,33	18,18	28,57	12,50	22,22	0,00	0,00	7,69	20,00	11,11	8,33	8,33	7,69	15,38	9,09	9,09	20,00
DPP	*	*	30,00	28,57	28,57	22,22	11,11	16,67	16,67	20,00	25,00	18,18	8,33	16,67	15,38	33,33	20,00	9,09
WH	*	*	*	40,00	27,27	23,08	25,00	20,00	35,71	22,22	50,00	50,00	28,57	35,71	42,86	54,55	30,77	41,67
LZ	*	*	*	*	42,86	50,00	22,22	33,33	36,36	16,67	57,14	40,00	40,00	36,36	33,33	44,44	30,00	30,00
YEL	*	*	*	*	*	50,00	37,50	14,29	36,36	0,00	57,14	55,56	16,67	36,36	45,45	30,00	30,00	44,44
ELD	*	*	*	*	*	*	44,44	11,11	41,67	0,00	30,00	33,33	23,08	21,43	28,57	36,36	36,36	25,00
PER	*	*	*	*	*	*	*	12,50	33,33	0,00	33,33	36,36	25,00	33,33	41,67	40,00	40,00	27,27
ZAR	*	*	*	*	*	*	*	*	30,00	25,00	28,57	33,33	20,00	30,00	16,67	22,22	10,00	10,00
SOL1	*	*	*	*	*	*	*	*	*	9,09	60,00	58,33	35,71	53,85	40,00	50,00	38,46	38,46
MIR	*	*	*	*	*	*	*	*	*	*	14,29	10,00	10,00	9,09	8,33	11,11	0,00	11,11
ARR	*	*	*	*	*	*	*	*	*	*	*	66,67	36,36	60,00	54,55	55,56	40,00	55,56
CAMP	*	*	*	*	*	*	*	*	*	*	*	*	28,57	58,33	53,85	41,67	30,77	54,55
LINE2	*	*	*	*	*	*	*	*	*	*	*	*	*	35,71	25,00	30,77	54,55	21,43
LAG	*	*	*	*	*	*	*	*	*	*	*	*	*	*	61,54	38,46	38,46	38,46
CAST	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	46,15	26,67	72,73
AGRO	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	45,45	45,45
FARR	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	23,08
CHI	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*

Figura 5 – Matriz de similaridade do padrão de bandas geradas por ITAP (Capítulo 3) com a combinação de primers **canônico** - **canônico**, em algumas linhagens de *Drosophila simulans*

<i>hobo¹⁻³</i> x <i>hobo¹⁻³</i>		RB	DPP	WH	LZ	YEL	ELD	PER	ZAR	SOL1	MIR	ARR	CAMP	LINE2	LAG	CAST	AGRO	FARR	CHI
RB	*	28,57	42,86	22,22	37,50	16,67	12,50	12,50	12,50	20,00	28,57	33,33	42,86	14,29	33,33	12,50	50,00	55,56	33,33
DPP	*	*	40,00	33,33	60,00	33,33	0,00	50,00	50,00	12,50	20,00	25,00	40,00	25,00	50,00	20,00	50,00	22,22	25,00
WH	*	*	*	50,00	50,00	25,00	16,67	16,67	16,67	25,00	40,00	50,00	60,00	20,00	42,86	16,67	75,00	33,33	20,00
LZ	*	*	*	*	66,67	20,00	33,33	14,29	37,50	37,50	14,29	16,67	28,57	0,00	57,14	14,29	33,33	30,00	0,00
YEL	*	*	*	*	*	20,00	33,33	33,33	33,33	37,50	33,33	40,00	50,00	16,67	83,33	33,33	60,00	44,44	16,67
ELD	*	*	*	*	*	*	0,00	0,00	0,00	16,67	0,00	0,00	25,00	0,00	16,67	0,00	33,33	12,50	0,00
PER	*	*	*	*	*	*	*	20,00	20,00	50,00	20,00	25,00	16,67	0,00	50,00	50,00	20,00	37,50	0,00
ZAR	*	*	*	*	*	*	*	*	12,50	20,00	25,00	16,67	25,00	50,00	50,00	20,00	22,22	25,00	0,00
SOL1	*	*	*	*	*	*	*	*	*	12,50	14,29	25,00	14,29	50,00	28,57	28,57	40,00	0,00	0,00
MIR	*	*	*	*	*	*	*	*	*	*	66,67	40,00	25,00	28,57	20,00	50,00	37,50	25,00	0,00
ARR	*	*	*	*	*	*	*	*	*	*	*	50,00	33,33	33,33	25,00	66,67	25,00	33,33	0,00
CAMP	*	*	*	*	*	*	*	*	*	*	*	*	20,00	42,86	16,67	75,00	33,33	20,00	0,00
LINE2	*	*	*	*	*	*	*	*	*	*	*	*	*	14,29	25,00	25,00	11,11	33,33	0,00
LAG	*	*	*	*	*	*	*	*	*	*	*	*	*	*	50,00	50,00	55,56	14,29	0,00
CAST	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	20,00	37,50	25,00	0,00
AGRO	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	37,50	25,00	0,00
FARR	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	11,11	0,00
CHI	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	0,00

Figura 6 – Matriz de similaridade do padrão de bandas geradas por ITAP (Capítulo 3) com a combinação de primers *hobo¹⁻³* – *hobo¹⁻³*, em algumas linhagens de *Drosophila simulans*.

CN x <i>hobo</i> ³		RB	DPP	WH	LZ	YEL	ELD	PER	ZAR	SOL1	MIR	ARR	CAMP	LINE2	LAG	CAST	AGRO	FARR	CHI	
RB	*	50,00	40,00	60,00	50,00	36,36	30,00	20,00	20,00	50,00	40,00	30,00	11,11	22,22	11,11	20,00	20,00	20,00	33,33	27,27
DPP	*		57,14	85,71	71,43	71,43	42,86	28,57	28,57	50,00	57,14	42,86	16,67	14,29	16,67	28,57	28,57	28,57	50,00	37,50
WH	*	*		50,00	57,14	37,50	28,57	33,33	33,33	57,14	66,67	28,57	20,00	16,67	20,00	33,33	33,33	33,33	60,00	42,86
LZ	*	*	*		62,50	62,50	37,50	25,00	25,00	62,50	50,00	37,50	14,29	28,57	14,29	25,00	25,00	25,00	42,86	33,33
YEL	*	*	*	*		50,00	25,00	28,57	28,57	50,00	57,14	25,00	16,67	14,29	16,67	28,57	28,57	28,57	50,00	22,22
ELD	*	*	*	*	*		66,67	28,57	33,33	33,33	57,14	66,67	16,67	14,29	16,67	28,57	12,50	28,57	57,14	
PER	*	*	*	*	*	*		40,00	25,00	25,00	50,00	100,00	25,00	20,00	25,00	40,00	16,67	40,00	50,00	
ZAR	*	*	*	*	*	*	*		*	28,57	60,00	40,00	33,33	25,00	33,33	100,00	50,00	50,00	33,33	
SOL1	*	*	*	*	*	*	*	*	*		57,14	25,00	16,67	33,33	16,67	28,57	50,00	50,00	22,22	
MIR	*	*	*	*	*	*	*	*	*	*		50,00	20,00	16,67	20,00	60,00	33,33	60,00	42,86	
ARR	*	*	*	*	*	*	*	*	*	*	*		25,00	20,00	25,00	40,00	16,67	40,00	50,00	
CAMP	*	*	*	*	*	*	*	*	*	*	*	*		50,00	100,00	33,33	33,33	33,33	20,00	
LINE2	*	*	*	*	*	*	*	*	*	*	*	*	*		50,00	25,00	25,00	25,00	16,67	
LAG	*	*	*	*	*	*	*	*	*	*	*	*	*	*		33,33	33,33	33,33	20,00	
CAST	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*		50,00	50,00	33,33	
AGRO	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	50,00	14,29
FARR	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	14,29
CHI	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*

Figura 7 – Matriz de similaridade do padrão de bandas geradas por ITAP (Capítulo 3) com a combinação de primers *canônico* – *hobo*³, em algumas linhagens de *Drosophila* *simulans*.