



UNIVERSIDADE ESTADUAL PAULISTA
“JÚLIO DE MESQUITA FILHO”
Câmpus de São José do Rio Preto

Bruna Emilia Roman

Infecção do endossimbionte *Wolbachia* em populações de *Drosophila sturtevanti* (Drosophilidae, grupo *saltans*, subgrupo *sturtevanti*): prevalência, efeito no valor adaptativo e aquisição da cepa

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Tese apresentada como parte dos requisitos para obtenção do título de Doutora em Biociências, junto ao Programa de Pós-Graduação em Biociências, do Instituto de Biociências, Letras e Ciências Exatas da Universidade Estadual Paulista “Júlio de Mesquita Filho”, Câmpus de São José do Rio Preto.

Financiadora: CAPES

Orientadora: Prof^a. Dr^a. Lilian Madi-Ravazzi

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2022

R758i	Roman, Bruna Emilia Infecção do endossimbionte Wolbachia em populações de <i>Drosophila sturtevantii</i> (Drosophilidae, grupo saltans, subgrupo sturtevantii) : prevalência, efeito no valor adaptativo e aquisição da cepa / Bruna Emilia Roman. -- São José do Rio Preto, 2022 163 p. : il., tabs., mapas Tese (doutorado) - Universidade Estadual Paulista (Unesp), Instituto de Biociências Letras e Ciências Exatas, São José do Rio Preto Orientadora: Lilian Madi Ravazzi 1. Incompatibilidade citoplasmática. 2. wStv. 3. Supergrupo A. 4. Transmissão horizontal. I. Título.
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São José do Rio Preto
09 de setembro de 2022

AGRADECIMENTOS

O presente trabalho é um esforço de mais de 4 anos de muito trabalho e dedicação, mas se cheguei até aqui, devo meus agradecimentos também às pessoas que permitiram de alguma forma que essa tese se concretizasse.

Agradeço primeiramente aos meus pais, Brás e Néia e à minha irmã Monique, que se dedicaram à minha educação e que sempre me incentivaram a estudar, por terem acreditado em mim desde sempre, pelo apoio fundamental, por cada oração, e pela compreensão ao serem privados em muitos momentos da minha companhia e atenção. E ao meu sobrinho Arthur que me trouxe muita alegria. Sem vocês, eu não teria chegado até onde cheguei!

À minha orientadora, Profa. Dra. Lilian Madi Ravazzi, por ter aceitado me orientar e me dado a oportunidade de trabalhar com as drosófilas e com a *Wolbachia* que eu tanto queria e por toda ajuda e suporte para que o projeto funcionasse.

Aos meus amigos que passaram pelo laboratório de *Drosophila* do IBILCE, Carol, Natália, Guilherme, Jéssica, Samara, Bruna, Segala, Gabriel e Larissa, por todos os momentos compartilhados, pelo bate papo no café da tarde, risadas, fofocas (do bem), pelo apoio nos momentos difíceis, pelas festinhas e por tudo que me ensinaram ao longo de minha trajetória.

Agradeço, em especial, à Samara por todos os ensinamentos durante o mestrado e início do doutorado, por todo o apoio, por tudo que fez por mim, dentro e fora do laboratório e claro por sua amizade que é muito valiosa. E agradeço, em especial também, à Carol, que trabalhou junto comigo, fez análises para mim, pelas valiosas discussões sobre meus dados, pelas soluções e orientação quando eu mais precisava, e por toda amizade fora do laboratório, festinhas e rolês.

À Nayla, por todo apoio e incentivo, que esteve presente nas minhas crises e dificuldades, pelo amor, companheirismo, pelas flores nas comemorações das conquistas e chocolates nos dias tristes. Agradeço também, à Susi, que me aconselhou e por toda conversa sobre a vida acadêmica.

Às minhas amigas de Rio Preto, Marina, Mari, Natália, Carol, Laura, Luana e Bruna, com quem passei a maior parte do meu tempo livre me divertindo, rindo, jogando conversa fora e me aconselhando. Ao Heitor e ao Pig e à todos os meus amigos de Urupês que sempre me acolheram, por todo apoio, por todas as conversas e pelos conselhos. À Mariane por todas as caronas e desabafos.

Aos meus amigos do vôlei da UNESP de Rio Preto que fizeram os meus dias mais felizes e leves.

À todos os colegas, funcionários e professores do Ibilce que de alguma forma contribuíram com a minha trajetória, tirando dúvidas e me auxiliando sempre que necessário. Em especial, ao Tião e à Natália pela ajuda com o meio de cultura e pelos pedidos realizados de última hora.

Aos professores Luciana Paes, Rogério Mateus, Lilian Castiglioni, Cláudia Carareto e aos colegas Maryanna e Willian que se disponibilizaram em me ajudar nas análises mais complicadas.

À Universidade Estadual Paulista “Júlio de Mesquita Filho” – UNESP, campus São José do Rio Preto, por ter aberto as portas nos últimos 7 anos, para que eu pudesse realizar toda minha pesquisa e pelo suporte, pelas amizades incríveis que fiz e pelo meu vôleizinho.

Ao Programa de Pós-graduação em Biociências pela oportunidade de realização da minha pesquisa e pelo auxílio financeiro, quando precisei.

E à todos que não mencionei aqui, mas que fizeram parte da minha trajetória, meus sinceros agradecimentos!

Agradeço à FAPESP pela concessão do auxílio fornecido ao meu grupo de pesquisa, sob o processo nº 2016/11994-5, Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP).

O presente trabalho foi realizado com apoio da Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES), Código de Financiamento 001.

“Science and everyday life cannot and should not be separated. Science, for me, gives a partial explanation of life. In so far as it goes, it is based on fact, experience and experiment.”

Rosalind Franklin, in a letter to her
father, summer 1940

RESUMO

Wolbachia é um gênero endossimbiótico de Alfabroteobactérias pertencentes à ordem Rickettsiales, que infecta cerca de 50% dos artrópodes e inúmeros nematoides. Muitas cepas causam manipulações reprodutivas em seus hospedeiros que favorecem a disseminação da infecção, como a incompatibilidade citoplasmática, morte dos machos, feminização e indução de paternogênese. A *Wolbachia* é transmitida principalmente verticalmente, da mãe para a prole, entretanto casos de transmissão horizontal, entre espécies, já foram relatados. Os hospedeiros, portanto, podem adquirir a *Wolbachia* por transmissão cladogênica, introgressiva ou ainda por transferência horizontal. O efeito da infecção e o modo de aquisição desse endossimbionte não foram muito bem estudados em muitos hospedeiros naturais. Dentre esses hospedeiros pouco estudados, se encontra a *Drosophila sturtevantii*, uma espécie neotropical, amplamente distribuída em domínios da Mata Atlântica e outros biomas. O presente trabalho buscou identificar a cepa de *Wolbachia* infectante de 14 populações de *D. sturtevantii*, avaliar a prevalência dessa infecção, analisar o efeito no valor adaptativo do hospedeiro e a indução de manipulação reprodutiva em cinco populações. Ainda, avaliar a forma de aquisição da *wStv*, por *D. sturtevantii*, e de outras cepas pertencentes ao supergrupo A, que infectam espécies do gênero *Drosophila*. A cepa *wStv* foi identificada como a única infectante nas populações de *D. sturtevantii*. A infecção apresentou taxas variáveis (0-70%) e prevalência média de 41%, significando que essa cepa não foi fixada nessas populações ainda. Foi observada uma ampla variação no efeito da *Wolbachia* no valor adaptativo das cinco populações de *D. sturtevantii* e também incompatibilidade citoplasmática parcial em duas populações. A variação da infecção observada, possivelmente corresponde ao background genético de cada população hospedeira. Foi observada também uma associação entre prevalência e efeito no valor adaptativo, as populações com maior prevalência apresentam mais efeitos benéficos e as com menor prevalência apresentam mais efeitos negativos. Esses resultados sugerem que a *wStv* esteja sendo mantida nas populações de *D. sturtevantii* devido aos efeitos benéficos e/ou neutros. Os nossos resultados ainda indicam que as aquisições de *wStv*, por *D. sturtevantii*, assim como as demais cepas pelas espécies do gênero *Drosophila* ocorreram por transmissão horizontal.

Palavras-chave: Incompatibilidade citoplasmática. *wStv*. Supergrupo A. Transmissão horizontal.

ABSTRACT

Wolbachia is an endosymbiotic genus of Alphaproteobacteria belonging to the order Rickettsiales, which infects about 50% of arthropods and many nematodes. *Wolbachia* is maternally transmitted from mother to offspring. Many strains cause reproductive manipulations in their hosts, which favors the spread of infection, such as cytoplasmic incompatibility, male-killing, feminization, and induction of parthenogenesis. *Wolbachia* is mainly transmitted vertically from mother to offspring, however cases of horizontal transmission, between species, have already been reported. Hosts, therefore, can acquire *Wolbachia* by cladogenic, introgression or horizontal transmission. The effect of infection and the way of acquisition of this endosymbiont has not been very well studied in many natural hosts. Among these poorly studied hosts is *Drosophila sturtevantii*, a neotropical species, widely distributed in areas of the Atlantic Forest and other biomes. The present work aimed to identify the *Wolbachia* strain infecting 14 populations of *D. sturtevantii*, assess the prevalence of this infection, analyze the influence on host fitness and the induction of reproductive manipulation in five populations. And to evaluate the way of acquisition of *wStv*, by *D. sturtevantii*, and other strains belonging to the supergroup A, which infect species of the genus *Drosophila*. The *wStv* strain was identified as the only one infecting the populations of *D. sturtevantii*. The infection showed variable rates (0-70%), and average prevalence of 41%, meaning that it has not been fixed in these populations yet. A wide variation in the effect of *Wolbachia* on the fitness of the five populations of *D. sturtevantii* and partial cytoplasmic incompatibility in two populations was observed. The variation of infection observed possibly corresponds to the genetic background of each host population. An association between prevalence and effect on fitness was also observed, in which populations with higher prevalence present more beneficial effects, and those with lower prevalence present more negative effects. These results suggest that *wStv* is being maintained in *D. sturtevantii* populations due to beneficial and/or neutral effects. Our results also indicate that the acquisition of *wStv*, by *D. sturtevantii*, as well as the other strains by the species of the genus *Drosophila* occurred by horizontal transmission.

Keywords: Cytoplasmic incompatibility. *wStv*. Supergroup A. Horizontal transmission.

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LISTA DE ABREVIATURAS E SIGLAS

AG	Aguaí
BET	Betary
BRA	Brasília
CANT	Cantareira
CI	Cytoplasmic incompatibility
COI	Cytochrome c oxidase subunit 1
COII	Cytochrome c oxidase subunit 2
CUR	Curió
DOU	Dourados
DCV	<i>Drosophila C</i> vírus
ELD	Eldorado
FHV	Flock House vírus
F1	First filial generation
GLM	Generalized linear model
GUA	Guaribas
IC	Incompatibilidade citoplasmática
MK	Male-killing
ML	Maximum likelihood
MLST	Multilocus Sequence Typing
mtDNA	DNA mitochondrial
mya	Million years ago
NG	Nova Granada
NCBI	National Center for Biotechnology Information
nDNA	DNA nuclear
PCR	Polymerase chain reaction
PER	Pernambuco
PI	Piraí
PIC	Picinguaba
PRA	Pratigi
RI	Ribeirão da Ilha
TIR	Tirimbina
VIT	Vitória

wsp	<i>Wolbachia</i> surface protein
wmk	WO-mediated killing

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1. INTRODUÇÃO

1.1. O endossimbionte *Wolbachia*

Wolbachia é um gênero endossimbiótico de alfa-proteobactérias, gram-negativas, que pertencem à família Anaplasmataceae e à ordem Rickettsiales (HERTIG; WOLBACH, 1924). *Wolbachia pipientis* é atualmente a única espécie descrita dentro desse gênero e por isso é comumente referida apenas como *Wolbachia* (NEWTON; SLATKO, 2019). Esse endossimbionte é o mais abundante no reino animal (TAYLOR et al., 2018), infecta cerca de 40% dos artrópodes terrestres e 52% dos artrópodes aquáticos (ZUG; HAMMERSTEIN, 2012; SAZAMA et al., 2017), é encontrada em isópodos (ROUSSET et al., 1992), em ácaros (JOHANOWICZ; HOY, 1995), e especialmente em insetos, infectando cerca da metade das espécies (WEINERT et al., 2015; ROSS et al., 2019). Além dos artrópodes, também é encontrada em várias espécies de nematoides (LEFOULON et al., 2016).

Wolbachia pipientis foi observada pela primeira vez em ovários do mosquito *Culex pipiens* (HERTIG; WOLBACH, 1924). Essa bactéria é comumente encontrada nos tecidos germinativos dos hospedeiros, a qual promove manipulações reprodutivas e efeitos diversos no valor adaptativo, resultando no aumento de sua transmissão na população hospedeira (MIN; BENZER, 1997). Além disso, esse endossimbionte também é encontrado nos tecidos somáticos dos hospedeiros, como por exemplo em *Drosophila*, a qual já foram observadas infecções no cérebro (intra e extracelular), retina, lobo óptico, gânglios, células-tronco somáticas, glândulas salivares, músculos, intestino médio, túbulos de Malpighi, asas, hemolinfa e em outros tecidos (DOBSON et al., 1999; VENETI et al., 2003; OSBORNE et al. 2009; CASPER-LINDLEY et al., 2011; ALBERTSON et al., 2013; STRUNOV et al., 2013; TOOMEY et al., 2013).

Inicialmente, a diversidade genética da *Wolbachia* foi caracterizada pelos marcadores 16S *rRNA* (gene ribossomal) (O'NEILL et al., 1992) e pelo *wsp* (gene de proteína de superfície de membrana), que apresenta regiões hipervariáveis (ZHOU et al., 1998), ambos foram muito importantes em análises populacionais e filogenéticas. E após, novos marcadores genéticos começaram a ser empregados, como é o caso do sistema *Multilocus Sequence Typing* (MLST) (BALDO et al., 2006). Atualmente, com o avanço da genômica comparativa, foi observado que, em muitos casos, somente dados do MLST não refletem suficientemente bem as propriedades de cada

cepa de *Wolbachia* (BLEIDORN; GERTH, 2018; WOLFE et al., 2021), necessitando o sequenciamento de todo o genoma. Os genes *16S rRNA*, *wsp*, os genes que compõe o MLST e os estudos genômicos recentes, foram amplamente utilizados na classificação do gênero *Wolbachia* em linhagens monofiléticas, geneticamente distintas que hoje são chamados de supergrupos (ZHOU et al., 1998).

Esse gênero é considerado geneticamente diverso, sendo dividido atualmente em 19 supergrupos (A-U) (LEFOULON et al., 2020; LAIDOUDI et al., 2020; KAUR et al., 2021; OLANRATMANEE et al., 2021), com exceção do supergrupo G, descrito em aranhas australianas (ROWLEY et al., 2004) e do supergrupo R, descrito em aranhas das cavernas (WANG et al., 2016), que foram realocadas como pertencentes aos supergrupos B e A, respectivamente (BALDO; WERREN, 2007; GERTH, 2016). Os supergrupos C, D e J são exclusivamente encontrados nos nematoides filarioides (BANDI et al., 1998; CASIRAGHI et al., 2004; LEFOULON et al., 2016). Já o supergrupo L ocorre em nematoides parasitas de plantas *Pratylenchus penetrans* (HAEGEMAN et al., 2009), enquanto o supergrupo F é o único clado composto por cepas infectantes tanto de artrópodes (térmitas), quanto de nematoides (*Mansonella ozzardi*) (CASIRAGHI et al., 2006). E os demais, sendo A e B mais comuns, encontrados na maioria dos artrópodes (VANDEKERCKHOVE et al., 1999; LO et al., 2007).

Ainda, dentro de cada supergrupo, existem cepas diferentes, comumente nomeadas de acordo com sua distribuição inicial ou hospedeiro infectado inicialmente, como por exemplo: a cepa *wHa* que infecta moscas no Hawaii (O'NEILL; KARR, 1990); a *wAu* que inicialmente foi encontrada na Austrália (TURELLI; HOFFMAN, 1995); *wMau* infectante da espécie *Drosophila mauritiana* (GIORDANO et al., 1995), além da cepa *wMel*, que é uma das mais estudadas e presente em *Drosophila melanogaster* (HOLDEN et al., 1993). Essa última cepa é diversa e se encontra subdividida em vários genótipos, como por exemplo, *wMel*, *wMel2*, *wMel3*, *wMelCS*, *wMelCS2* (RIEGLER et al., 2005). Cada cepa pode atuar de maneira diferente em cada hospedeiro, resultando em diferentes efeitos na ecologia e evolução do hospedeiro (GRUNTENKO et al., 2017).

Um único hospedeiro pode estar infectado com mais de um tipo de cepa (coinfecção), assim como hospedeiros de uma mesma espécie podem apresentar infecções por cepas diferentes ou ainda hospedeiros de espécies diferentes podem apresentar infecção por um mesmo tipo de cepa (transmissão horizontal) (WERREN

et al., 2000; MERÇOT; CHARLAT, 2004; RIEGLER et al.; 2005). A prevalência das cepas nos hospedeiros é altamente variável (WEINERT et al., 2015), ocorrendo em taxas baixas e intermediárias de prevalência (HOFFMANN et al., 1996; GIORDANO et al., 1995; CHARLAT et al., 2004; CATTEL et al., 2016; MEANY et al., 2019), que comumente estão correlacionadas com possíveis eventos estocásticos, com a falta de incompatibilidade citoplasmática (IC), IC incompleta, e transmissão maternal imperfeita (HOFFMANN et al., 1990; CHAMPION DE CRESPIGNY et al., 2005). E também ocorrendo em altas taxas de prevalência, que leva a fixação das cepas nas populações hospedeiras, e normalmente estão relacionadas com a capacidade de manipulações reprodutivas e efeitos benéficos dessas cepas aos hospedeiros (WERREN, 1997; KRIESNER et al., 2013).

1.2. Interação *Wolbachia*-hospedeiro

A *Wolbachia* pode manipular o sistema reprodutivo de seus hospedeiros resultando na maioria das vezes, no aumento da sua taxa de transmissão, gerando resultados diretos sobre a razão sexual das populações, seja por meio da indução da partenogênese, feminização, morte dos machos, e a mais comum, a incompatibilidade citoplasmática (IC) (LAVEN, 1951; YEN; BARR, 1971; TURELLI; HOFFMANN, 1991; ROUSSET et al., 1992; STOUTHAMER et al., 1993; HURST; JIGGINS, 2000). Na indução da partenogênese, fêmeas virgens produzem prole composta por fêmeas, por meio de óvulos não fertilizados (STOUTHAMER et al., 1993; KAUR et al., 2021). Nessa manipulação, a *Wolbachia* atua em organismos haplodiploides transformando machos em fêmeas. Nestes organismos, normalmente, os machos se desenvolvem a partir de ovos haploides não fertilizados (partenogênese arrenótoca), enquanto as fêmeas se desenvolvem a partir de ovos diploides fertilizados (partenogênese telítoca) (KAUR et al., 2021). A *Wolbachia* então age duplicando o número de cromossomos dos ovos haploides não fertilizados, convertendo-os em diploides, o que resulta na formação de fêmeas produzidas assexuadamente (STOUTHAMER et al., 1999). Essa manipulação ocorre comumente na ordem Hymenoptera (ZCHORI-FEIN et al., 1992; MEYER; HOY, 2007; BOIVIN et al., 2014), mas também já foi observada em espécies de ácaros (WEEKS et al., 2001).

Em organismos diploides, a feminização ocorre por meio da ação hormonal, transformando machos genéticos em fêmeas fenotípicas e funcionais (ROUSSET et

al., 1992; KAGEYAMA et al., 1998). Algumas linhagens de *Wolbachia* conseguem causar a feminização em seu hospedeiro por meio da ação sobre a glândula androgênica, nas quais as bactérias proliferam-se, resultando em hipertrofia e inibição da sua função (AZZOUNA et al., 2004). Como consequência, as fêmeas infectadas passam a produzir o dobro de fêmeas em relação às não infectadas, aumentando também a frequência de *Wolbachia* nos hospedeiros (CHARLAT et al., 2003). Essa manipulação é uma das mais raras e foi descrita pela primeira vez em isópodes (LEGRAND et al., 1984), mas também ocorre em insetos das ordens Hymenoptera, Lepidoptera e em aranhas (NEGRI et al., 2012; CURRY et al., 2015).

O fenótipo morte dos machos ou “*male-killing*” (MK) ocorre quando a *Wolbachia* mata seletivamente os machos infectados da prole, frequentemente ainda na embriogênese, que resulta em uma distorção na razão sexual, tendenciosa para as fêmeas. Essa distorção sexual é frequentemente alta, próxima de 100% de prole composta por fêmeas (HURST; JIGGINS, 2000; DYER; JAENIKE, 2004; RICHARDSON et al., 2022). Esse fenótipo ocorre em organismos, como coleópteros, lepidópteros e aracnídeos (pseudoescorpíões) e também já foi relatado em espécies de *Drosophila* (HURST; JIGGINS, 2000; FIALHO; STEVENS, 2000; JIGGINS et al., 2001; DYER; JAENIKE, 2004; ZEH et al., 2005, RICHARDSON et al., 2022).

Estudos genômicos recentes identificaram um possível gene candidato, na *Wolbachia*, pela indução de MK, denominado de *wmk* (*WO-mediated killing*), que é um regulador transcricional putativo, que está localizado na região EAM do prófago WO (PERLMUTTER et al., 2019). Desta forma, a expressão desse gene pode causar a letalidade de machos ainda na embriogênese, associada a danos ao DNA, assim como defeitos citológicos, como pontes de cromatina no cromossomo X, núcleos picnóticos e falha mitótica (KAUR et al., 2021). Apesar da MK causar a diminuição do número de prole, essa manipulação favorece as fêmeas infectadas em relação às não infectadas, visto que elas passam a se alimentar de seus irmãos mortos (ovos não eclodidos), ocorrendo também a redução da competição entre os descendentes (ZEH et al., 2005) e redução da endogamia (WERREN, 1997). Assim, a *Wolbachia* passa a se espalhar nas populações hospedeiras, visto que MK favorece as fêmeas infectadas e a transmissão é via materna (JAENIKE et al., 2003).

A manipulação reprodutiva mais comum induzida por *Wolbachia*, a IC, foi observada pela primeira vez no mosquito *Culex pipiens*, em que ocorriam cruzamentos incompatíveis, com poucos ou nenhum descendente gerado, sendo

estes resultados associados a um padrão de herança citoplasmática (LAVEN, 1951; GHELELOVITCH, 1952). Em 1971, Yen e Barr conseguiram eliminar a bactéria *Wolbachia* das linhagens de *C. pipiens*, por meio de antibióticos, observando a produção normal de descendentes e reversão da IC (YEN; BARR, 1971). Essa manipulação reprodutiva pode ocorrer de duas formas, de maneira unidirecional, que é caracterizada quando fêmeas não infectadas cruzam com machos infectados, ou ainda, bidirecionalmente, quando as fêmeas infectadas cruzam com machos infectados por cepa de *Wolbachia* diferente da sua, resultando na diminuição ou ausência da prole (WERREN, 1997). Assim, a infecção por *Wolbachia* pode fornecer vantagem ao hospedeiro no aumento da progênie, e ao mesmo tempo proporciona um local para a sua propagação (O'NEILL et al., 1997; WERREN et al., 2008).

Os níveis de manipulação em relação a IC, podem diferir entre as cepas de *Wolbachia* e seus hospedeiros, resultando em pouco ou nenhum efeito (HOLDEN et al., 1993; SOLIGNAC et al., 1994; GIORDANO et al., 1995; HOFFMANN et al., 1996; MIN; BENZER, 1997; CHARLAT et al., 2004; CATTEL et al., 2016; SAEED et al., 2018; MEANY et al., 2019). Além disso, foi observado que, em algumas espécies de *Drosophila*, a IC é mais forte em machos mais jovens, logo que o fator de IC é expresso em níveis mais baixos em machos com mais idade (REYNOLDS; HOFFMANN, 2002; LEPAGE et al., 2017).

Alguns mecanismos foram propostos para explicar em nível citológico como a *Wolbachia* pode induzir IC, mas algumas questões ainda continuam em aberto. Um destes mecanismos envolve a incapacidade de condensação dos cromossomos paternos nas divisões celulares mitóticas do zigoto quando o espermatozoide fecunda um óvulo não infectado, resultando em células aneuploides (WERREN, 1997; STOUTHAMER et al., 1999; MERÇOT; POINSOT, 2009). Quando a fêmea estiver infectada pela mesma cepa de *Wolbachia* que o macho, alterações também ocorrerão nas fêmeas, podendo resgatar as modificações provocadas nos cromossomos paternos para completar a cariogamia e desenvolver normalmente o embrião (LASSY; KARR, 1996; CALLAINI et al., 1997). Porém, se a fêmea não estiver infectada ou estiver infectada, mas, por uma cepa diferente do macho, tais modificações provocadas nos espermatozoides não serão recuperadas, bloqueando a sua descendência (MCGRAW et al., 2002).

Recentemente, foi observado que após o acasalamento com machos infectados por *Wolbachia*, as fêmeas não infectadas de *D. melanogaster* mostraram

respostas fisiológicas pós-cópula alteradas quando comparadas às fêmeas acasaladas com machos não infectados (HE et al., 2018). Isto poderia ser devido à redução da expressão de algumas proteínas do fluido seminal induzidas pela infecção por *Wolbachia* (HE et al., 2018). Outro mecanismo sugerido propõe que os espermatozoides em desenvolvimento podem ser modificados por algum fator, ainda desconhecido, cuja secreção é induzida por *Wolbachia* presente nas células germinativas dos machos infectados (BECKMANN et al., 2017; LEPAGE et al., 2017). Quando ovos que trazem a mesma linhagem de *Wolbachia* são fertilizados por tais espermatozoides modificados, um fator de ligação complementar derivado de *Wolbachia* resgata o efeito da modificação, o qual, de outra maneira, levaria ao aumento da mortalidade embrionária (KRIESNER; HOFFMANN, 2018).

Em nível genético, estudos recentes com as cepas *wMel* (*D. melanogaster*) e *wPip* (*C. pipiens*) demonstraram que os genes bacterianos *cifA* e *cifB* são os responsáveis pela indução de IC (BECKMANN et al. 2017; LEPAGE et al. 2017; CHEN et al. 2019; SHROPSHIRE; BORDENSTEIN 2019). Nos genomas estudados, os genes *cifA* e *cifB* são encontrados pareados dentro de um prófago chamado WO, e é discutido que ambos são, provavelmente, transcritos como um único operon (BECKMANN et al. 2017; CHEN et al. 2019). O modelo genético que explica essa manipulação via esses genes é chamado de *Two-by-One*, pois em experimentos com *D. melanogaster*, os espermatozoides dos machos infectados são modificados pela expressão do *cifA* e *cifB* nas linhagens germinativas masculinas (LEPAGE et al., 2017; SHROPSHIRE; BORDENSTEIN 2019; SHROPSHIRE et al. 2020). Mas, quando fêmeas infectadas cruzam com machos infectados pelas mesmas cepas, a IC pode ser resgatada pela expressão do gene *cifA*, na linhagem germinativa feminina (SHROPSHIRE et al., 2018; SHROPSHIRE; BORDENSTEIN 2019). Portanto, o mecanismo de IC ocorre pela expressão de ambos os genes para a modificação dos espermatozoides, mas apenas um para o resgate (SHROPSHIRE; BORDENSTEIN 2019).

Os genes *cif* são encontrados em várias cópias nos genomas de *Wolbachia*, e ainda podem ser frequentemente adquiridos por transmissão horizontal, o que pode explicar os padrões mais complexos de incompatibilidade (LINDSEY et al., 2018; BONNEAU et al., 2018). Ainda, é sugerido que esses homólogos evoluem sob pressões evolutivas diferentes do restante do genoma de *Wolbachia* e por isso, foi observado por meio de análises filogenéticas utilizando as proteínas Cif, que o CifA e

CifB possuem filogenia semelhantes, onde co-divergem em cinco clados filogenéticos, denominados de Tipos 1-5 (LEPAGE et al. 2017; SHROPSHIRE et al., 2020).

A interação entre *Wolbachia* e seu hospedeiro resulta em um “multiorganismo”, ou seja, ambos compartilham fisiologia, desenvolvimento, comportamento e história evolutiva, de tal modo que além das manipulações reprodutivas, a *Wolbachia* pode influenciar o valor adaptativo do hospedeiro, e esse relacionamento *Wolbachia*-hospedeiro pode se tornar obrigatório, no qual o hospedeiro passar a ser totalmente dependente desse endossimbionte, por promover funções essenciais (MORAN et al., 2008; FERRARI; VAVRE, 2011; FLATAU et al., 2021). Um exemplo desse caso ocorre em percevejos, a qual a *Wolbachia* passou a fornecer vitaminas do complexo B, que são essenciais para o crescimento e reprodução desses percevejos (HOSOKAWA et al. 2010). Também existem os casos em que os hospedeiros não são dependentes da *Wolbachia*, mas esta, por sua vez, fornece fenótipos benéficos aos hospedeiros, que acaba se tornando um tipo de interação facultativa, ou seja, mutualismo facultativo (ZUG; HAMMERSTEIN, 2015). Nesse tipo de interação a *Wolbachia* promove efeitos vantajosos ao valor adaptativo do hospedeiro, como resistência a inseticidas (DURON et al., 2006); geração de ATP para o hospedeiro (DARBY et al. 2012); contribuição para sobrevivência em baixas temperaturas (BYKOV et al., 2019); aumento da taxa de eclosão dos ovos e da viabilidade (SAEED et al., 2018); aumento da proliferação de células troncos (FAST et al., 2011), proteção antiviral (HEDGES et al. 2008; TEIXEIRA et al. 2008; OSBORNE et al., 2009; UNCKLESS; JAENIKE, 2012; CHROSTEK et al. 2013; MARTINEZ et. Al., 2014; KRIESNER; HOFFMANN, 2018), entre outros.

A proteção antiviral foi inicialmente descoberta na cepa wMel em *D. melanogaster*, a qual foi possível observar uma redução da carga viral nos hospedeiros infectados, fazendo com que eles sobrevivam por mais tempo do que os não infectados (HEDGES et al. 2008; TEIXEIRA et al. 2008). Esse tipo de efeito promovido pela *Wolbachia* sob o hospedeiro, passou a ser utilizado como controle biológico, logo que em mosquitos, a *Wolbachia* consegue limitar a replicação de vários vírus nos tecidos somáticos do hospedeiro, reduzindo assim a transmissão da infecção para os humanos (WALKER et al., 2011; ALIOTA et al., 2016; DUTRA et al., 2016; PEREIRA et al., 2018). Desta forma, a cepa wMel, de *D. melanogaster* passou a ser introduzida em mosquitos como o *Aedes aegypti*, vetor da dengue, que não são naturalmente infectados por *Wolbachia*, resultando assim na redução e até supressão

da transmissão desse vírus (HOFFMANN et al., 2011; WALKER et al., 2011). Essa cepa também foi utilizada como controle de outros vírus, como o da chikungunya (ALIOTA et al., 2016; TAN et al., 2017), do zika vírus (ALIOTA et al., 2016; DUTRA et al., 2016; TAN et al., 2017), do vírus da febre amarela (VAN DEN HURK et al., 2012) e até o vírus do Nilo Ocidental (HUSSAIN et al., 2013). Ainda, existem outros estudos que foram e ainda estão sendo desenvolvidos com outras cepas encontradas em outras espécies de hospedeiros, para controle biológico, como a cepa *wPip*, para o controle do vírus do Nilo Ocidental (GLASER; MEOLA, 2010) e a cepa *wAlbB* que é utilizada para bloquear a replicação de flavivírus e alphavirus (EKWUDU et al., 2020).

A *Wolbachia* também pode promover o aumento da fecundidade das fêmeas hospedeiras infectadas, em relação às não infectadas, como já foi observado em algumas espécies de *Drosophila*, como em *D. paulistorum* (MILLER et al., 2010), *D. innubila* (UNCKLESS; JAENIKE, 2012), *D. simulans* (KRIESNER et al., 2013) e em *D. melanogaster* (SERGA et al., 2014). E também pode aumentar a longevidade dos hospedeiros (FRY; RAND, 2002; BRUMMEL et al., 2004; ALEXANDROV et al., 2007; HE et al., 2018; CAPOBIANCO III et al., 2018). Brummel et al. (2004) observaram que algumas moscas do gênero *Drosophila* não infectadas apresentaram uma menor longevidade quando comparadas com algumas moscas infectadas por *Wolbachia*, sugerindo que a presença de comunidade simbiótica favorece uma maior expectativa de vida. Além do mais, de alguma forma o endossimbionte *Wolbachia* é capaz de afetar, em alguns casos, a expressão de certos genes associados à longevidade em *Drosophila* (MAISTRENKO et al., 2016). Genes estes que são responsáveis por vias e processos fundamentais para viabilidade destas moscas, como resposta imune, metabolismo energético, defesa contra estresse oxidativo, entre outros (MAISTRENKO et al., 2016).

Além dos fenótipos mutualistas aqui mencionados, a *Wolbachia* ainda pode induzir fenótipos parasitas, resultando em efeitos negativos para o hospedeiro (ZUG; HAMMERSTEIN, 2015). Em espécies de *Drosophila*, já foram observados efeitos negativos na longevidade e fecundidade dos hospedeiros (MIN; BENZER, 1997; FRY; RAND, 2002; BRUMMEL et al., 2004; HE et al., 2018; CAPOBIANCO III et al., 2018; SAEED et al., 2018), alterações negativas no comportamento de cópula (MARKOV et al., 2009; SHARON et al., 2010; MILLER et al., 2010; HE et al., 2018), degradação dos tecidos nervosos e musculares (MIN; BENZER, 1997), entre outros.

Ainda, pode ser que a *Wolbachia* não cause manipulações reprodutivas, como a IC, nem provoque efeitos perceptíveis aos hospedeiros, persistindo em frequências mais baixas, se comportando nas populações hospedeiras como variantes neutras (GIORDANO et al., 1995; HOFFMANN et al., 1996; CHARLAT et al., 2004; KRIESNER et al., 2013). No entanto, é esperado que mesmo infecções neutras para manipulações reprodutivas, consigam se manter nas populações hospedeiras, promovendo o mínimo de efeitos benéficos a elas ou ainda que tenha uma maior eficiência na transmissão materna (GIORDANO et al., 1995). Charlat et al. (2004), ao estudarem populações naturais de *D. yakuba* infectadas por *Wolbachia*, não encontraram evidências de manipulação reprodutiva, como IC e nem alterações da razão sexual, relacionando esses resultados a um comportamento neutro da *Wolbachia*, mas que a manutenção desse endossimbionte ocorre devido a transmissão materna perfeita (100% de prevalência). A não indução de IC por *Wolbachia* tem sido observada em outras espécies hospedeiras, como Meany et al., (2019), ao estudar a cepa wMau em *D. mauritiana*, não encontraram evidências dessa manipulação e relacionaram a frequência intermediária encontrada a um possível efeito positivo no valor adaptativo não identificado.

1.3. Transmissão da *Wolbachia*

A transmissão é um dos principais processos intimamente relacionados ao sucesso no estabelecimento do endossimbionte nas populações hospedeiras. O principal meio de transferência da *Wolbachia* é por via materna, da mãe para a prole, chamada de transmissão vertical a partir do citoplasma do ovo (WERREN, 1997). No entanto, existem casos em que a transmissão vertical é imperfeita, ou seja, o endossimbionte não consegue atingir a transmissão ideal e apresenta falhas na dispersão e no seu estabelecimento no hospedeiro (JAENIKE, 2009; TURELLI; HOFFMANN, 1995). Um dos fatores que permite que a transmissão vertical tenha sucesso é a densidade em equilíbrio da *Wolbachia* no hospedeiro (FUNKHOUSER-JONES et al., 2018; LÓPEZ-MADRIGAL; DUARTE, 2020), pois quando ocorre infecção em baixa densidade, pode resultar na perda do endossimbionte em oogônias em desenvolvimento, reduzindo assim a sua transferência para próxima geração (WERREN, 2005; JAENIKE, 2009). Já quando ocorre o inverso, a infecção em altas densidades, pode ser que a *Wolbachia* gere efeitos parasitários ao hospedeiro, como

por exemplo, reduzindo a longevidade em *Drosophila* (MIN; BENZER, 1997), reduzindo a fecundidade em vespas (CHAFEE et al., 2011) ou ainda levando a morte do hospedeiro (SERBUS et al., 2011).

Somente a transmissão vertical não esclarece a ampla distribuição desse endossimbionte nos artrópodes e nematoides. Associada a essa questão, foi observado que as filogenias de algumas cepas apresentavam incongruências com as filogenias dos hospedeiros, sendo assim, uma forma de transmissão passou a ser investigada, a transmissão horizontal ou *host shift* (mudança de hospedeiro) (O'NEILL et al., 1992). Esse tipo de transmissão pode ocorrer dentro de uma mesma espécie (transmissão horizontal intraespecífica) (SU et al., 2019) ou ainda entre espécies diferentes (transmissão interespecífica), até filogeneticamente distantes, como por exemplo, entre artrópodes e nematoides (GERTH et al., 2014; GERTH; BLEIDORN, 2016; TURELLI et al., 2018; SCHOLZ et al., 2020). Vários são os fatores que contribuem para que a *Wolbachia* consiga ser transmitida entre espécies diferentes (horizontalmente), como a sua sobrevivência no meio extracelular, mesmo sendo um endossimbionte intracelular obrigatório, a facilidade de se mover pelos tecidos e células dos hospedeiros (tropismo), sua alta facilidade em se recombinar e de se adaptar a genomas e ambientes diversos (TOLLEY et al., 2019; SANAEI et al., 2020; STRUNOV et al., 2022). Para o entendimento das transmissões não maternas pelos hospedeiros, as rotas ecológicas constituem uma importante questão.

As rotas da transmissão horizontal podem ocorrer via um vetor ou diretamente, via exposição ao ambiente externo (HEATH et al., 1999; CORDAUX et al., 2001; BALDO et al., 2008; AHMED et al., 2015; LI et al., 2017; SANAEI et al., 2020). Por exemplo, já foi observado uma possível fonte de transmissão de *Wolbachia* via canibalismo entre larvas de *Drosophila* (HAINE et al., 2005). Há poucos casos relatados da transmissão da *Wolbachia* entre presa e predadores (SANAEI et al., 2020). Mas a transmissão via parasitismo é mais frequente, logo que o parasita pode ser encontrado em vários estágios e em vários tecidos dos seus hospedeiros, aumentando as chances de transferência da *Wolbachia* entre por essa via (SANAEI et al., 2020). Pode ser que uma única espécie parasitoide seja fonte de *Wolbachia* para múltiplos hospedeiros ou ainda que os parasitoides sejam apenas os vetores entre as espécies hospedeiras, sem ser infectados (NODA et al., 2001; DEDEINE et al., 2005; AHMED et al., 2015). A transmissão via parasitoides já foi observada em espécies de *Drosophila*, onde encontraram cepas de *Wolbachia* idênticas (ou

similares), entre *Drosophila* e vespas parasitoides (Hymenoptera) (VAVRE et al., 1999). Esse tipo de rota é frequentemente especulado nos trabalhos de transmissão horizontal entre espécies de *Drosophila* (MILLER; RIEGLER, 2006; METCALF et al., 2014). Os ácaros, que são ectoparasitas de *Drosophila*, são possíveis vetores na transmissão da *Wolbachia* entre as espécies de *Drosophila*. Já foi observado em estoques de laboratório, que os ácaros brancos da espécie *Tyrophagus putrescentiae* podem se infectar com a *Wolbachia* ao se alimentarem de cadáveres de *Drosophila* infectados e, de forma contrário, as larvas de *Drosophila*, podem se infectar com *Wolbachia* ingerindo ácaros mortos infectados (BROWN; LLOYD, 2015).

Nichos compartilhados, como as fontes de alimentos, podem ser ótimos ambientes para propagação da *Wolbachia*, visto que já foi observado a presença desse simbiote nas glândulas salivares de insetos herbívoros e não herbívoros, sendo essa bactéria transferida pelo contato do aparato bucal dos hospedeiros com os ambientes externos (DOBSON et al., 1999; SANAEI et al., 2020). As plantas são exemplos dessas plataformas ecológicas, as quais já foram observadas que várias cepas de *Wolbachia* sobreviveram dentro de plantas por longos períodos, facilitando a sua transferência para diversos hospedeiros (BURKE et al., 2020). Outro estudo demonstrou que as plantas conhecidas como feijão-de-corda e o pepino mediam a transmissão horizontal entre as moscas-brancas *Bemisia tabaci* infectadas para as não infectadas e que a *Wolbachia* persistiu em vasos do floema por pelo menos 50 dias (LI et al., 2017). Estudos como esses demonstram que espécies diversas de artrópodes que se alimentam das mesmas plantas podem compartilhar as mesmas cepas de *Wolbachia*, como já foi observado em uma plantação de abóboras (SINTUPACHEE et al., 2006). No caso das espécies de *Drosophila*, os seus estágios imaturos são saprófitos e se alimentam de leveduras sobre o material vegetal em decomposição e como visto, esse nicho é compartilhado por inúmeros microrganismos, como a *Wolbachia*, podendo esse ambiente ser uma de rota de transmissão para as espécies de *Drosophila* também (THROCKMORTON, 1975; MATEOS et al., 2006). Além de plantas já foi observado também outras fontes de alimentos, como fungos entre formigas que crescem em fungos com seus parasitas sociais (TOLLEY et al., 2019).

Ainda pode ser que as transmissões ocorram diretamente por contato, como é verificado em cupins do gênero *Cubitermes*, para o qual foi sugerido que a transferência de *Wolbachia* ocorreu por meio de trocas de secreções salivares,

durante a trofalaxia (ROY et al., 2015). Já foi observado, em isópodes, a transmissão horizontal via hemolinfa entre indivíduos feridos, infectados e não infectados (RIGAUD; JUCHAULT, 1995). Entre esses e outros exemplos, é possível concluir que a movimentação da *Wolbachia* pelo tecido somático do hospedeiro é um dos principais fatores para que ocorra a transmissão horizontal (PIETRI et al., 2016).

A análise de como as cepas foram adquiridas é muito importante para responder questões evolutivas e ecológicas dos hospedeiros, mas estudos como esses, sobre o modo de aquisição, assim como o tempo de divergência, foram pouco relatados na literatura (O'NEILL et al., 1992; ROUSSET; SOLIGNAC 1995; HUIGENS et al., 2004; BALDO et al., 2008; RAYCHOUDHURY et al., 2009; AHMED et al., 2015; TURELLI et al., 2018). A aquisição de *Wolbachia* pelo hospedeiro pode ocorrer de três formas: por aquisição cladogênica, por introgressão e por aquisição horizontal, como já mencionado anteriormente (TURELLI et al., 2018). Na aquisição cladogênica, espécies irmãs herdam a cepa de *Wolbachia* do ancestral comum recente, durante a especiação, como observado em vespas e abelhas (RAYCHOUDHURY et al., 2009; GERTH; BLEIDORN, 2016). A aquisição por introgressão e hibridação, ocorre quando as espécies filogeneticamente próximas hibridizam, adquirindo a cepa da espécie irmã (RAYCHOUDHURY et al., 2009; ROUSSET; SOLIGNAC, 1995). A aquisição introgressiva pode ser considerada mais comum em espécies do gênero *Drosophila*, visto que há relatos de diversas espécies intimamente relacionadas ocorrendo em faixas geográficas sobrepostas e consequentemente podendo hibridizar (COYNE; ORR, 1989, 1997; GARRIGAN et al., 2012; TURELLI et al., 2014).

É possível estudar a forma de aquisição das cepas por meio da comparação das filogenias e estimativa de tempo de divergência entre os genomas de *Wolbachia* e do hospedeiro, tanto do DNA mitocondrial (mtDNA), quanto do DNA nuclear (nDNA) (RAYCHOUDHURY et al. 2009; CONNER et al. 2017; TURELLI et al. 2018). Quando há concordância entre as filogenias e estimativas de tempo de divergência entre os três genomas, sugere-se a aquisição cladogênica (CONNER et al. 2017; TURELLI et al. 2018; COOPER et al., 2019). Quando apenas há concordância entre as filogenias e estimativas de tempo de divergência entre a *Wolbachia* e o mtDNA, suporta a aquisição introgressiva (CONNER et al. 2017; TURELLI et al. 2018; COOPER et al., 2019). E quando não há concordância entre nenhuma filogenia e estimativa de tempo de divergência, é sugerido que a *Wolbachia* tenha sido adquirida horizontalmente (CONNER et al. 2017; TURELLI et al. 2018; COOPER et al., 2019).

1.4. Associação *Wolbachia* e mtDNA do hospedeiro

As manipulações reprodutivas, favorecem a dispersão da *Wolbachia* que pode ocorrer de forma rápida, também influenciando na diversidade e frequência de haplótipos do mtDNA nas populações hospedeiras (HURST; JIGGINS, 2005), logo que esse endossimbionte e as mitocôndrias são transmitidos da mãe para prole, sendo co-herdados maternalmente (TURELLI; HOFFMANN, 1991, 1995; HOFFMAN et al., 1998). A influência pode ocorrer se a *Wolbachia* for co-transmitida maternalmente em conjunto com o mtDNA, em desequilíbrio de ligação, no entanto, caso ocorra transferência horizontal ou paternal em frequência suficiente, em ambos, o desequilíbrio de ligação pode ser interrompido, não havendo essa correlação (TURELLI et al., 1992; HURST; JIGGINS, 2005).

Para melhor exemplificar a associação da *Wolbachia* e do mtDNA, foi observado em populações de *D. simulans*, da Califórnia, originalmente não infectadas, que a partir dos anos de 1980, uma cepa de *Wolbachia* passou a infectá-las, resultando na indução da incompatibilidade citoplasmática (IC) (TURELLI; HOFFMANN, 1991). Essa cepa conseguiu se espalhar rapidamente nas populações, carregando indiretamente um haplótipo de mtDNA que antes era raro ou ausente em populações não infectadas (TURELLI et al., 1992). Esses exemplo demonstra que cada cepa de *Wolbachia* pode estar associada a um haplótipo mitocondrial da população hospedeira, a qual, a partir do momento que a infecção conseguir se estabelecer e se espalhar, o mtDNA associado à infecção inicial pegará “carona” na população, observando assim uma seleção indireta do mtDNA; esse fenômeno é chamado de varredura seletiva (KAMBHAMPATI et al., 1992; HURST; JIGGINS, 2005). Desta forma, alguns tipos de manipulações do sistema reprodutivo do hospedeiro, como a IC, influenciam diretamente na diversidade do mtDNA, o conduzindo a essa varredura seletiva (HURST; JIGGINS, 1995).

De acordo com Hurst e Jiggins (2005), a influência deste simbiote no mtDNA do hospedeiro ocorre dependendo do tempo de invasão e do número de simbioses presentes na população, podendo resultar na redução ou aumento da diversidade de mtDNA e na variação da distribuição de frequências de haplótipos na população. Em populações naturais de diversos hospedeiros, casos onde a *Wolbachia* foi associada com a redução da diversidade do mtDNA dos hospedeiros já foram relatados (JIGGINS, 2003; RAYCHOUDHURY et al., 2010; GRAHAM; WILSON, 2012; XIAO et

al., 2012, SIGNOR, 2017). Como visto, o mtDNA de populações infectadas por esse tipo de endossimbionte, pode ser afetado, portanto a utilização de somente dados de sequências de mtDNA, pode ser considerada inadequada para estudos evolutivos em artrópodes (HURST; JIGGINS, 1995; SMITH et al., 2012).

1.5. *Drosophila sturtevantii* e a cepa wStv

Existem vários estudos sobre a interação de *Wolbachia* com espécies do gênero *Drosophila*, entretanto, a maioria com *D. melanogaster* e *D. simulans*, permanecendo desconhecidos os efeitos de muitas cepas de *Wolbachia* infectantes de outras espécies do gênero *Drosophila*. Em relação à *Drosophila sturtevantii*, objeto do presente estudo, há poucos estudos sobre os efeitos da infecção de *Wolbachia* para esta espécie. A *D. sturtevantii* (grupo *saltans*, subgrupo *sturtevantii*, MAGALHÃES; BJÖRNBERG, 1957) é a espécie mais abrangente dentro do grupo *saltans* de *Drosophila*, e sua distribuição geográfica na região neotropical, estende-se desde o México tropical até o sul do Brasil, e também em alguns pontos no México, na região neártica (MAGALHÃES, 1962). Sua ocorrência foi relatada em diferentes biomas e apresenta grande abundância populacional em diferentes épocas do ano, sendo, portanto, uma espécie adequada para diversos tipos de estudos, com destaque para os evolutivos (MAGALHÃES, 1962; MATA et al., 2008; PENARIOL; MADI-RAVAZZI, 2013; TRAVA et al., 2021). Diversos estudos populacionais e evolutivos já foram realizados com *D. sturtevantii*, tais como polimorfismo cromossômico (DOBZHANSKY; PAVAN, 1943; KOBAYASHI; BICUDO, 1997a), estudo dos componentes do valor adaptativo (CARARETO; MOURÃO, 1992; CARARETO et al., 1999), isolamento sexual (DOBZHANSKY, 1944), genética de populações (TADEI; MOURÃO, 1981), isolamento reprodutivo (KOBAYASHI; BICUDO, 1997b), elementos de transposição (ALMEIDA; CARARETO, 2002; DE SETTA et al., 2007), morfometria de asas e edeagos (SEGALA, 2019), estrutura populacional a partir de microssatélites espécie-específicos (TRAVA et al., 2021) e filogeografia utilizando genes mitocondriais (ZORZATO et al., 2022).

Os últimos trabalhos publicados por nosso grupo de pesquisa têm encontrado estruturação populacional dessa espécie, como por exemplo, Segala (2019), observou por meio da morfometria das asas e dos edeagos, estruturação latitudinal que reflete diferenciação genética entre as populações estudadas. Trava et al. (2021) observaram

estrutura genética moderada para nove populações do Brasil, a partir dos resultados com microssatélites espécie-específicos. No trabalho de Zorzato et al. (2022) com análises filogeográficas, utilizando os genes mitocondriais *COI* e *COII*, para 21 populações geográficas de *D. sturtevantii*, das quais 16 são as mesmas utilizadas no presente estudo, foi observado estruturação genética entre as populações, com a formação de três haplogrupos, associados às variáveis geográficas e climáticas. Além disso, também foi verificado uma alta diversidade haplotípica e baixa diversidade nucleotídica, e esse tipo de variação do mtDNA pode estar correlacionado com a infecção de múltiplas cepas de *Wolbachia* nas populações hospedeiras (HURST; JIGGINS, 1995). Uma das questões levantada por Zorzatto et al. (2022) foi se a distribuição dos haplótipos mitocondriais encontrada estaria relacionada com as variáveis geográficas e climáticas, ou com a infecção por *Wolbachia* dessas populações hospedeiras, logo que a interação da *Wolbachia* com o hospedeiro pode resultar em uma seleção indireta do mtDNA e na variação da distribuição de frequências de haplótipos na população hospedeira.

A cepa *wStv* que infecta naturalmente a espécie *D. sturtevantii* foi pouco estudada. O primeiro estudo com *D. sturtevantii* e a associação com *Wolbachia* foi realizado por Bourtzis et al. (1996). Estes autores testaram a presença da infecção em 10 amostras de *D. sturtevantii*, do Stock Center de Bowling Green (EUA), com os *primers* *dnaA* e *16S rRNA*, e não verificaram a infecção pela bactéria (BOURTZIS et al., 1996). Por outro lado, em 2006, Miller e Riegler, ao analisarem oito linhagens de *D. sturtevantii*, identificaram pela primeira vez a cepa *wStv*, em quatro delas, e além disso, dentre as linhagens infectadas, foram encontradas três variantes: *wStv-MI*, *wStv-Pan6* e *wStv-SG*. Nesse mesmo trabalho, a cepa *wStv* foi caracterizada como pertencente ao supergrupo A, mas filogeneticamente distante das demais cepas infectantes de *Drosophila*, também pertencentes ao mesmo supergrupo (MILLER; RIEGLER, 2006). Mateos et al. (2006) testaram a presença da infecção de *Wolbachia* em várias espécies do gênero *Drosophila*, incluindo *D. sturtevantii*, os quais foram analisados sete indivíduos vindos de Stock Center de Tucson (EUA), e apenas dois apresentaram a infecção pela cepa *wStv-MI*, a mesma encontrada no trabalho de Miller e Riegler (2006). Até então, apenas a prevalência havia sido avaliada para essa cepa. No trabalho de Martinez et al. (2014), foi avaliada a proteção antiviral conferida pela *Wolbachia* ao hospedeiro, para dois vírus de RNA: *Flock House virus* (FHV) e *Drosophila C virus* (DCV). Esse estudo foi realizado com 19 cepas de *Wolbachia*

infectantes de 16 espécies de *Drosophila*, entre elas a cepa *wStv* de *D. sturtevantii*. Para controlar os efeitos genéticos dos hospedeiros, todas as cepas foram transferidas artificialmente para a espécie *D. simulans*. Os resultados mostraram que a cepa *wStv* oferece proteção antiviral contra ambos os vírus (FHV e DCV), para o hospedeiro artificial *D. simulans* (MARTINEZ et al., 2014). Ainda é interessante destacar que a densidade de *Wolbachia* no hospedeiro, foi importante para determinar o nível de proteção antiviral, a qual *wStv* foi observada em alta densidade (MARTINEZ et al., 2014).

A cepa *wStv* infectante de *D. sturtevantii* e outras 15 cepas de *Drosophila* foram utilizadas, novamente por meio da transinfecção em *D. simulans*, para o estudo sobre a correlação entre o fenótipo da incompatibilidade citoplasmática (IC) e a proteção antiviral (MARTINEZ et al., 2015). Esses estudos demonstraram que não há correlação entre ambos os fenótipos, mas que uma forte proteção antiviral por requerer altas densidades da infecção no tecido somático do hospedeiro, promove reduções substanciais de outras características do valor adaptativo do hospedeiro. Em relação a cepa *wStv*, foi observado que ela induz simultaneamente a IC e a proteção antiviral no hospedeiro artificial *D. simulans*, além disso, também foi observada a diminuição do tempo de vida de fêmeas infectadas com *wStv*, mas não foi correlacionada com a proteção antiviral (MARTINEZ et al., 2015).

Recentemente, foram iniciadas análises genômicas com a cepa *wStv*. Martinez et al. (2021), estudaram a evolução dos genes *cif* (*cifA* e *cifB*), que são envolvidos com a indução da IC, nos genomas de várias espécies de *Drosophila*, incluindo *D. sturtevantii*. Esse estudo demonstrou que o genoma de *wStv* apresenta dois pares homólogos dos genes e que em um desses pares (par 1), foram encontradas mutações putativas de perda de função, tanto para o gene *cifA* (uma deleção - T(A)TTTTAG>TTTTTAG), como para o gene *cifB* (uma substituição - CAG>TAA e uma inserção de um elemento de transposição). Portanto, esses eventos sugerem que um dos pares dos genes *cif* pode ter sofrido pseudogenização e perdido assim a função de induzir IC, enquanto o outro permanece sem mutações (MARTINEZ et al., 2021).

Recentemente, Strunov et al. (2022) analisaram o tropismo em tecidos somáticos e reprodutivos de seis cepas infectantes de *Drosophila*, entre elas a *wStv*. Os autores encontraram resultados semelhantes no padrão de infecção nos ovários e no cérebro para as cepas *wStv*, *wPau* (*D. paulistorum*), *WWil* (*D. willistoni*) e *wLeh* (*D.*

lehrmanae), espécie recentemente descrita (MADI-RAVAZZI, et al., 2021) e incluída no mesmo subgrupo de *D. sturtevantii*. Baseado nesse padrão encontrado e na filogenia das cepas, os autores sugeriram que um último ancestral comum mais recente de *D. sturtevantii* e *D. lehrmanae*, possivelmente carregou a cepa ancestral wAu-like, esta que por sinal é intimamente relacionada às cepas wWil e wPau e a muitas outras cepas infectantes de *Drosophila* (STRUNOV et al., 2022). Ainda, foi sugerido, que essa cepa ancestral (wAu-like), quando estava infectando a espécie ancestral de *D. sturtevantii* e *D. lehrmanae* foi eliminada por uma cepa ancestral similar a wStv-like, presente hoje tanto em *D. sturtevantii* como em *D. lehrmanae*, que era considerada mais agressiva e que com o passar do tempo evolutivo se tornou domesticada (STRUNOV et al., 2022).

Algumas questões permanecem em aberto sobre a cepa wStv, uma delas é sobre a sua influência no valor adaptativo e manipulação reprodutiva em populações naturais, como é o caso de *D. sturtevantii*, seu hospedeiro natural, e também como ocorreu a sua forma de aquisição por *D. sturtevantii*, comparada com outras cepas infectantes de espécies do grupo *saltans* de *Drosophila* ou mesmo do gênero *Drosophila*. Além dessas, outras questões gerais sobre esse simbiote e sua relação com o hospedeiro devem ser exploradas, principalmente devido a sua importância no controle de doenças transmitidas por vetores (WALKER et al., 2011; DUTRA et al., 2016; O'NEILL et al., 2019) e também no controle biológico de pragas na agricultura (BOURTZIS, 2008; NIKOLOULI et al., 2018; MATEOS et al., 2020).

2. OBJETIVOS

2.1. Objetivo geral

Avaliar a prevalência e o efeito da infecção da *Wolbachia* nas populações geográficas de *D. sturtevantii*, assim como o modo de aquisição das cepas do supergrupo A infectantes de espécies do gênero *Drosophila*.

2.2. Objetivos específicos

1. Analisar a presença da infecção por *Wolbachia*, em indivíduos recém coletados de populações geográficas de *D. sturtevantii*;
2. Identificar as cepas de *Wolbachia* nas populações de *D. sturtevantii*;
3. Avaliar a prevalência da *Wolbachia* nas populações de *D. sturtevantii*;
4. Analisar os efeitos da infecção por *Wolbachia* no valor adaptativo das populações de *D. sturtevantii*;
5. Avaliar a indução de manipulações reprodutivas pela *Wolbachia* nas populações de *D. sturtevantii*;
6. Avaliar o modo de aquisição das cepas do supergrupo A infectantes das espécies do grupo *saltans*, tais como *D. sturtevantii* (wStv), *D. lehrmanae* (wLeh), *D. prosaltans* (wPro), *D. pseudosaltans* (wPsd), *D. septentriosaltans* (wSpt) e de outras espécies do gênero *Drosophila*.

3. CAPÍTULO I

***Wolbachia* infection in natural populations of *Drosophila sturtevantii*: effects on fitness and reproductive manipulation**

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ACKNOWLEDGEMENTS

We thank Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) for the BER's PhD scholarship and Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) for the financial support (Number processes: 2014/14059-0 and 2016/ 11994-5). We thank the Conselho Nacional de Desenvolvimento Científico e Tecnológico for CP's PhD scholarship (CNPq 141545/2020-8).

CONFLICT OF INTEREST

The authors declare no conflict of interest.

Abstract

Wolbachia pipientis is the only species of the genus *Wolbachia*, belonging to the order Rickettsiales, which infects approximately 50% of arthropods species and many nematodes. Many strains cause reproductive manipulations in their hosts, which favors the spread of infection. The influence of this endosymbiont in many natural hosts, such as *Drosophila sturtevantii*, a neotropical species widely distributed in domains of the Atlantic Forest and other biomes, has not been well studied. The objective of the present work was to identify the *Wolbachia* strain infecting 14 populations of *D. sturtevantii*, to evaluate the prevalence of this infection and to analyze its effect on the fitness and reproductive manipulation of the host. The wStv strain was identified as the only infective strain in the *D. sturtevantii* populations. The infection frequency showed variable rates (0-70%), with a mean prevalence of 41%. Variation in the effect of infection on host fitness and cytoplasmic incompatibility induction was verified, which may be related to the genetic background of each host population. An association was also found between the prevalence of *Wolbachia* and the effects on host fitness, in which the populations with higher prevalence showed higher fitness and those with lower prevalence had lower fitness for some parameters. Furthermore, *Wolbachia* does not appear to affect development time in any of the populations, and male-killing (MK) reproductive manipulation was not observed. Based on these results, we suggest

that the wStv strain is being maintained in populations due to potential beneficial and neutral effects on host fitness.

KEYWORDS: Cytoplasmic incompatibility, fecundity, prevalence, wStv

1. INTRODUCTION

Wolbachia is the most abundant intracellular endosymbiont in the animal kingdom (Taylor et al., 2018), infecting arthropods and nematodes (Zug & Hammerstein, 2012; Lefoulon et al., 2016; Sazama et al., 2017). Half of the insect species are estimated to be infected by this bacterium (Weinert et al., 2015; Ross et al., 2019). *Wolbachia* is maternally transmitted; however, there are cases of horizontal transmission, which may occur intra- and interspecies (Werren, 1997; Turelli et al., 2018; Su et al., 2019; Scholz et al., 2020). This bacterium can manipulate host reproduction to favour the increase in its transmission, either by inducing parthenogenesis (Rousset et al., 1992; Stouthamer et al., 1993), feminization of genetic males (Rousset et al., 1992), or male-killing (MK), in which infected females produce strongly female-biased sex ratios (Hurst & Jiggins, 2000). Cytoplasmic incompatibility (CI) is the most common reproductive manipulation, is characterized by incompatible crossing between uninfected females and infected males or even females infected by strains different from infected males, resulting in the reduction or absence of offspring (Yen & Barr, 1971; Turelli & Hoffmann, 1991). The induction of CI is explained by the two-by-one genetic model, which occurs when, in males, the *cifA* and *cifB* genes are expressed, resulting in the modification of the host sperm, and the rescue of this modification can occur when, in females, the *cifA* gene is expressed (Lepage et al., 2017; Shropshire & Bordenstein, 2019; Shropshire et al., 2020). The manipulations that have already been observed in *Drosophila* species are only CI and MK (Hamm et al., 2014). Reproductive manipulations are dependent on the genetic background of both *Wolbachia* strains and hosts (Zabalou et al., 2008; Veneti et al., 2012).

Wolbachia can cause positive/beneficial, neutral, and negative effects on host fitness. The beneficial effects caused by *Wolbachia* include antiviral protection (Walker et al., 2011; Martinez et al., 2014; Pereira et al., 2018; Cogni et al., 2021), increased fecundity (Kriesner et al., 2013; Serga et al., 2014), and increased longevity (Alexandrov et al., 2007; He et al., 2018; Capobianco III et al., 2018), among others. However, negative effects from *Wolbachia* infection have already been reported, such as decreased egg hatching rate, decreased fecundity, and longevity, among others (Sumida et al., 2017; Lopez et al., 2018). *Wolbachia* may not cause reproductive manipulations, nor does *Wolbachia* cause perceptible effects to the hosts, persisting at lower frequencies, behaving in host populations as neutral variants (Giordano et al., 1995; Hoffmann et al., 1996; Charlat et al., 2004; Kriesner et al., 2013). However, even infections that are neutral for reproductive manipulations are expected to manage to remain in host populations, promoting minimal beneficial effects to them or even having greater efficiency in maternal transmission (Giordano et al., 1995; Charlat et al., 2004; Kriesner et al., 2018). In addition to these effects, *Wolbachia* can also interact indirectly with the mitochondrial DNA (mtDNA) of host populations, once both are transmitted from the mother to the offspring, being coinherited maternally (Turelli & Hoffmann, 1991, 1995; Hoffmann et al., 1998). The presence of *Wolbachia* strains that spread easily may promote indirect selection of mitochondrial haplotypes from hosts through selective sweep (Kambhampati et al., 1992; Hurst & Jiggins, 2005).

The *Wolbachia*-host interaction has not been extensively investigated in nature, and there are few recently collected studies that evaluate the effect of infection in natural populations of hosts, in which strains generated by introgression or transinfection are often used (Teixeira et al., 2008; Martinez et al., 2014; Hamm et al., 2014). The use of these strains may not reveal how *Wolbachia* interacts with its hosts in nature, which may lead to incorrect conclusions about *Wolbachia*-host coevolution. The effects of *Wolbachia* infection on natural populations of *D. sturtevantii* are not yet known. This neotropical species of the *saltans* group, the *sturtevantii* subgroup (Magalhães, 1957), has the widest geographic distribution, occurring from tropical Mexico to southern Brazil and at some other points in Mexico in the Nearctic region (Magalhães, 1962). In addition, this species occurs in different biomes and has a high population abundance at different times of the year; therefore, it is a suitable species for different types of evolutionary studies (Magalhães, 1962; Mata et al., 2008; Trava et al., 2021). The present work demonstrated that the influence of the wStv strain on

fitness and reproductive manipulations interacts according to the genotype of each host (populations of *D. sturtevantii*) and that the prevalence is related to the effects caused by *Wolbachia* on fitness.

2. MATERIALS AND METHODS

2.1. *D. sturtevantii* field collections and laboratory lines

The populations used in the present study were collected from 2015 to 2018 in different geographic regions of Brazil and Central America. Once collected, the male individuals were identified and kept in 70% alcohol for later DNA extraction (Table 1). Isofemale lines were established from inseminated females recently collected for the TIR (Biological Reserve of Tirimbina, Costa Rica), CUR (Floresta do Curió, Fortaleza, CE, Brazil), VIT (Parque Estadual da Fonte Grande, Vitória, ES, Brazil), CANT (Parque Estadual da Cantareira, SP, Brazil) and ELD (Eldorado do Sul, RS, Brazil) populations, and individuals from these isofemale lines were used to evaluate the presence of infection and perform crosses (Table 1).

TABLE 1. Populations and GPS location used in this study, along with the number of males collected from the field and number of isofemale lines and respective codes. + = infected lines, – = uninfected lines.

Population	Population Codes	Latitude	Longitude	No. From field	No. Isofemale lines	Isofemale codes used in crosses
Reserva Biológica de Tirimbina, Sarapiquí, Costa Rica	TIR	10.41658	-84.12454	-	12	TIR04–, TIR10+, TIR11+, TIR14+
Floresta do Curió, Fortaleza, CE, Brazil	CUR	-3.82809	-38.46953	-	10	CUR16–, CUR06+, CUR08+, CUR42+
Reserva Ecológica Guaribas, Mamanguape, PB, Brazil	GUA	-6.73099	-35.14779	11	-	-
Pernambuco, PE, Brazil	PER	-8.81371	-36.9541	10	-	-
APA do Pratigi/Igrapiúna, BA, Brazil	PRA	-13.82052	-39.1423	15	-	-
Parque Estadual da Fonte Grande, Vitória, ES, Brazil	VIT	-20.30905	-40.34126	-	15	VIT05–, VIT01+, VIT06+, VIT10+
Parque Estadual da Serra do Mar, Picinguaba, SP, Brazil	PIC	-23.36316	-44.82446	15	-	-
Parque Estadual da Cantareira, SP, Brazil	CANT	-23.45428	-46.63602	-	21	CANT1–, CANT3+, CANT4+, CANT5+
Fazenda São João, Nova Granada, SP, Brazil	NG	-20.60939	-49.29085	10	-	-
Reserva Betary, Iporanga, SP, Brazil	BET	-24.58808	-48.62885	10	-	-
Reserva Ecológica do IBGE, Brasília, DF, Brazil	BRA	-15.94821	-47.87846	6	-	-
Dourados, MS, Brazil	DOU	-22.22802	-54.81307	7	-	-
Piraí, Joinville, SC, Brazil	PI	-26.27479	-48.94933	10	-	-
Ribeirão da Ilha, Florianópolis, SC, Brazil	RI	-27.71458	-48.56062	10	-	-
Aguaí Santuário Ecológico, Siderópolis, SC, Brazil	AG	-28.61392	-49.55711	10	-	-
Eldorado do Sul, RS, Brazil	ELD	-30.00028	-51.31189	-	14	ELD11–, ELD06+, ELD15+, ELD21+

2.2. DNA extraction and *Wolbachia* detection

DNA extraction of individual flies was performed using the Wizard Genomic DNA Purification Kit (Promega, Madison, WI, USA) following the manufacturer's instructions. DNA quality was tested by cytochrome c oxidase subunit 1 (*COI*) gene amplification (Table S1). *Wolbachia* detection in populations of *D. sturtevantii* was performed by polymerase chain reaction (PCR) using specific primers from the *Wolbachia* surface protein gene (*wsp*) (Table S1), which generate fragments from 590 to 632 bp (ZHOU et al., 1998). PCR was performed in a total volume of 25 µL containing 0.5 U of Taq DNA polymerase (Class Five), 1 mM deoxynucleoside triphosphate (dNTP), 400 nM primers, 1x buffer, 3 mM MgCl₂ and 15 ng of DNA. Thermocycling conditions were an initial cycle of 2 minutes at 95 °C; 35 cycles of 1 minute at 95 °C (DNA denaturation), 1 minute at 59 °C (annealing of the primers), 1 minute at 72 °C (extension) and, finally, 10 minutes at 72 °C. PCR amplification products were visualized on a 2% agarose gel.

2.3. Identification and analysis of *Wolbachia* strain genetic variability in *D. sturtevantii* populations

Based on initial analyses, the 16S *rRNA* gene was considered a potentially polymorphic marker for these populations of *D. sturtevantii* and was used for identification and analysis of genetic variability. The primers used are listed in Table S1. From each of the populations infected by *Wolbachia*, three wild males for most populations and two females and one male for TIR, CUR, VIT, CANT and ELD populations, were used to identify and analyse the genetic variability. PCR conditions were performed in the same way as described in Section 2.2. The samples were sequenced using a 3130xl sequencer (Applied Biosystems, Foster City, CA, USA) with the same primers and amplification conditions at the Laboratório Multiusuário Centralizado para Sequenciamento de DNA em Larga Escala e Análise de Expressão Gênica at the UNESP Campus in Jaboticabal. The sequences were aligned in the MAFFT program (Katoh & Standley, 2013). The search for identity was performed by basic local alignment search tool (BLAST) in the GenBank National Center for Biotechnology Information (NCBI) database.

2.4. Cross experiments

Five seven-day-old virgin females were allowed to mate with five seven-day-old virgin males in each of the three replicates of each cross analysed. The flies were transferred to new tubes with *Drosophila* culture medium (banana and *Saccharomyces cerevisiae*) three times, with an interval of three days. After this period, the parents were removed. The crosses were kept at a constant temperature of 22 ± 1 °C. The objective was to analyse reproductive parameters such as fecundity, longevity, and development time, as well as reproductive manipulations such as cytoplasmic incompatibility (CI) and male killing (MK).

Five populations of *D. sturtevantii* were used for these experiments: TIR, CUR, VIT, CANT and ELD. These populations were chosen because they represent three distinct geographical and haplotypic groups: the northern (TIR and CUR), central (VIT and CANT) and southern (ELD) groups, as previously evaluated by our research group (Zorzato et al., 2022) through a study of mitochondrial DNA. Except for the CANT population, which was collected in 2016, the others (CUR, ELD, TIR and VIT) were collected in 2018.

In each of these populations, four isofemale lines were selected, three naturally infected and one uninfected. Furthermore, four types of crosses were established and studied in each population: infected females x infected males (IN x IN), uninfected females x uninfected males (UN x UN), infected females x uninfected males (IN x UN) and uninfected females x infected males (UN x IN). The types of crosses and isofemale lines used are described in Table S2.

2.5. Fecundity and *Wolbachia* phenotypes

The fecundity was analysed by counting the number of all male and female offspring (F1) produced between the infected crosses (IN x IN) and the uninfected crosses (UN x UN) during 40 days from the beginning of the crosses. During those days, F1 was withdrawn to avoid crosses that generate F2.

For the CI analysis, the number of offspring that emerged during 40 days from the beginning of the crosses was compared between the “incompatible” cross type, which occurs between uninfected females and infected males (UN x IN), and the other compatible crosses (IN x UN; IN x IN; UN x UN).

To assess whether *Wolbachia* induces male-killing in *D. sturtevantii* populations, the difference in the sex ratio of the offspring produced by the types of crosses of infected females (IN x IN and IN x UN) with the control of uninfected females (UN x UN) was observed.

2.6. Longevity

For each of the five populations, 10 virgin males and 10 virgin females were collected from F1 from the control crosses (IN x IN and UN x UN), kept individually in tubes containing *Drosophila* culture medium (banana and *Saccharomyces cerevisiae*), and observed until their death. The flies were evaluated daily to verify the date of mortality. During the experiment, the flies were kept in a constant temperature chamber at 22 ± 1 °C and transferred to tubes containing fresh culture medium weekly.

2.7. Development time

The development time of the control crosses (IN x IN and UN x UN) was evaluated from the average number of days from the oviposition period until the emergence of the imagos of each replicate of each cross within each population.

2.8. Statistical analyses

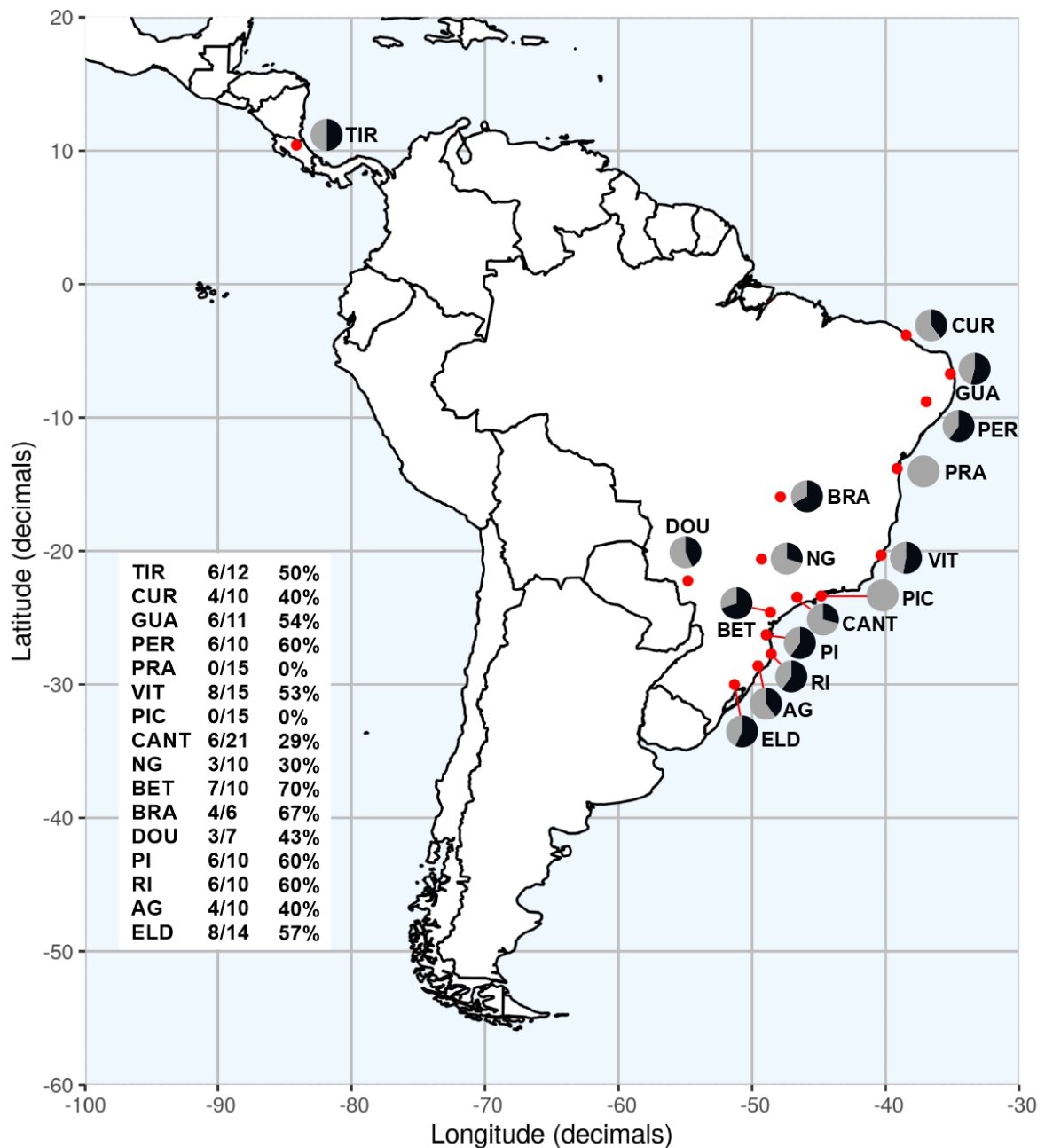
The dataset was analysed by the generalized linear model (GLM). The parameter response variable was fitted to the Poisson distribution with a log link function. Data were analysed at the 95% confidence interval in the R program (R Core Team, 2021). The experimental design was factorial, where the factors were type of cross between females and males and sex of F1. In the GLM, the omnibus test was used to assess whether there was a statistically significant difference from the complete model, which includes the type of crosses and sex as factors and compares it with the model that presented only the intercept. Probability p values smaller than 0.05 ($p < 0.05$) show that there is a statistically significant difference between the two types of models. GLM was evaluated by the Wald chi-squared test and probability p value. A pairwise comparison between the levels of each factor was performed using the Bonferroni test at a significance level of 5%.

3. RESULTS

3.1. Prevalence of *Wolbachia* infection in the *D. sturtevantii* populations

Of the 186 individuals tested for *D. sturtevantii* populations, 77 (41%) showed *Wolbachia* infection. Of the 16 populations evaluated, two were not infected (PRA and PIC), and the infection rate ranged from 0% to 70% (Figure 1).

FIGURE 1. *Wolbachia* prevalence of 16 populations of *D. sturtevantii* (see populations code in Table 1). Black = percentage of infected; Gray = percentage of uninfected



3.2. Identification and analysis of *Wolbachia* strain genetic variability in *D. sturtevantii* populations

The identification of the sequences obtained from *Wolbachia* resulted in 100% sequence identity with the *wStv* strain, which was previously found in *D. sturtevantii* (CP050531.1) (Martinez et al., 2021). No genetic variability of *wStv* was observed in the populations analysed by the *16S rRNA* gene. The strain already described in the literature was identified as belonging to supergroup A; thus, the *wStv* sequence observed in the *D. sturtevantii* populations analysed in the present work can be included in this supergroup.

3.3. Fecundity and *Wolbachia* phenotypes

The fecundity of the populations showed great variation, with total averages ranging from 26 individuals (UN x UN from VIT) to 148 (IN x UN from CUR) (Table 2). Significant differences were observed for the populations of CANT and CUR, in which the crosses of the uninfected (UN x UN) were more fertile than the crosses of the infected (IN x IN). For the TIR and VIT populations, the crosses of the infected (IN x IN) were more fertile than the crosses of the uninfected (UN x UN). For the ELD population, no significant difference was observed (Table S3).

In the evaluation of cytoplasmic incompatibility (CI), in the CANT population, the “incompatible” cross (UN x IN) showed the second highest mean for fecundity (126 individuals) and presented a significant difference only with the control cross of the uninfected ones (UN x UN); however, when compared to the other compatible crosses, there was no difference, suggesting that CI does not occur in this population. In the CUR population, the UN x IN cross showed the lowest fecundity average (71), showing significant differences from all other compatible crosses (Table 2, Table S3). In this case, a reduction of offspring produced in the “incompatible” cross is observed, but not abruptly, suggesting a weak induction of CI (partial CI) for the CUR population. A similar result was obtained for VIT, in which the “incompatible” cross showed low fecundity (55) and a significant difference with the IN x UN and IN x IN crosses but presented a higher mean than the UN x UN control cross (26) (Table 2); therefore, it seems to be a possible induction of weak CI here as well. The ELD and TIR

populations, however, showed high fecundity for the UN x IN cross; in this case, there was no indication of CI (Table 2, Table S3).

In the analysis of male-killing (MK) reproductive manipulation, all types of crosses, such as infected females (IN x IN and IN x UN), as well as the UN x UN control, showed a greater number of emerged females than males. However, significant differences were observed only for the crossings of infected females, such as the IN x IN cross for the CANT, TIR and VIT populations and the IN x UN type for the CUR, TIR and VIT populations. Although a female-biased sex ratio was observed, the ratio was not strong (close to 100%), so there was no indication of MK in these populations (Table S3).

3.4. Longevity

In terms of longevity, considerable variation was also observed, ranging from 72 days of life (females from UN x UN in ELD) to 141 days (males from UN x UN in CUR) (Table 2). A significant difference was observed for the CANT population, in which uninfected females and males had a longer lifespan than infected ones. For the ELD population, a significant difference was also observed, but contrary to the CANT result, infected females and males demonstrated longer lifespans than uninfected ones. For the TIR population, a significant difference was also observed, in which the infected males demonstrated a longer lifespan than the uninfected males and in the case of females, the uninfected demonstrated a longer lifespan than the infected females. The CUR and VIT populations did not show significant differences (Table S3).

3.5. Development time egg-to-imago

For the development time, the results were homogeneous, with a mean variation between 16.06 days of ovo-imago development (IN x IN of VIT) and 18.92 days (UN x UN of CANT), with no significant difference (Table 2, Table S3).

TABLE 2. Mean values of emerged adults (fecundity), longevity and development time analyzed for each type of cross of each population of *D. sturtevantii* studied. $\pm se$ = standard error; UN = uninfected; IN = infected, F = female; M = male; T = total.

Population	Type of Cross	Mean emerged adults ($\pm se$)				Mean longevity ($\pm se$)		Mean development time ($\pm se$)
		N	F	M	T	F	M	
Parque Estadual da Cantareira, SP, Brazil	UN x UN	3	82.33 $\pm$ 5.24	66.00 $\pm$ 4.69	148.33 $\pm$ 7.03	130.78 $\pm$ 3.81	140.90 $\pm$ 3.75	18.92 $\pm$ 1.27
	IN x IN	9	65.78 $\pm$ 2.70	52.44 $\pm$ 2.41	118.22 $\pm$ 3.62	105.47 $\pm$ 1.87	129.00 $\pm$ 2.07	16.74 $\pm$ 0.69
	UN x IN	9	67.11 $\pm$ 2.73	59.56 $\pm$ 2.57	126.67 $\pm$ 3.75	-	-	-
	IN x UN	9	61.56 $\pm$ 2.61	51.89 $\pm$ 2.40	113.44 $\pm$ 3.55	-	-	-
Floresta do Curió, Fortaleza, CE, Brazil	UN x UN	3	62.67 $\pm$ 4.57	45.33 $\pm$ 3.89	108.00 $\pm$ 6.00	132.56 $\pm$ 3.84	141.00 $\pm$ 3.75	17.58 $\pm$ 1.21
	IN x IN	9	46.71 $\pm$ 2.58	39.57 $\pm$ 2.38	86.29 $\pm$ 3.51	134.22 $\pm$ 2.42	131.30 $\pm$ 2.39	17.88 $\pm$ 0.86
	UN x IN	6	38.33 $\pm$ 2.53	32.50 $\pm$ 2.33	70.83 $\pm$ 3.44	-	-	-
	IN x UN	6	84.25 $\pm$ 4.59	64.25 $\pm$ 4.01	148.5 $\pm$ 6.09	-	-	-
Eldorado do Sul, RS, Brazil	UN x UN	3	50.00 $\pm$ 4.08	39.33 $\pm$ 3.62	89.33 $\pm$ 5.46	72.00 $\pm$ 2.68	102.60 $\pm$ 3.20	18.73 $\pm$ 1.30
	IN x IN	9	45.22 $\pm$ 2.24	37.22 $\pm$ 2.03	82.44 $\pm$ 3.03	104.69 $\pm$ 1.90	121.00 $\pm$ 2.01	18.84 $\pm$ 0.77
	UN x IN	6	58.50 $\pm$ 3.82	54.75 $\pm$ 3.70	113.25 $\pm$ 5.32	-	-	-
	IN x UN	6	27.83 $\pm$ 2.15	27.67 $\pm$ 2.15	55.50 $\pm$ 3.04	-	-	-
Reserva Biológica de Tirimina, Sarapiquí, Costa Rica	UN x UN	3	37.00 $\pm$ 4.30	30.00 $\pm$ 3.87	67.00 $\pm$ 5.79	122.11 $\pm$ 3.68	86.20 $\pm$ 2.94	17.56 $\pm$ 1.40
	IN x IN	9	51.33 $\pm$ 2.39	35.89 $\pm$ 2.00	87.22 $\pm$ 3.11	108.40 $\pm$ 1.90	114.63 $\pm$ 1.95	16.97 $\pm$ 0.69
	UN x IN	9	53.43 $\pm$ 2.76	36.71 $\pm$ 2.29	90.14 $\pm$ 3.59	-	-	-
	IN x UN	9	48.00 $\pm$ 2.45	34.25 $\pm$ 2.07	82.25 $\pm$ 3.21	-	-	-
Parque Estadual da Fonte Grande, Vitoria, ES, Brazil	UN x UN	3	17.00 $\pm$ 2.38	9.33 $\pm$ 1.76	26.33 $\pm$ 2.96	103.22 $\pm$ 3.39	131.30 $\pm$ 3.62	16.63 $\pm$ 1.44
	IN x IN	9	46.11 $\pm$ 2.26	32.89 $\pm$ 1.91	79.00 $\pm$ 2.96	94.07 $\pm$ 1.77	119.41 $\pm$ 2.03	16.06 $\pm$ 0.69
	UN x IN	6	33.83 $\pm$ 2.38	65.89 $\pm$ 71.23	55.50 $\pm$ 3.04	-	-	-
	IN x UN	6	54.00 $\pm$ 3.00	35.83 $\pm$ 2.44	89.83 $\pm$ 3.87	-	-	-

3.6. Correlation of effects on fitness and prevalence of *Wolbachia* in *D. sturtevantii* populations

In the results presented, there was a tendency for populations with lower prevalence to present more negative than beneficial effects and populations with higher prevalence to present more beneficial effects for some of the reproductive parameters evaluated (Table 3). The population of CANT showed negative effects, such as decreased fecundity and longevity of females and males infected in relation to uninfected individuals, and a neutral effect on development time. Associated with these results, CANT had the lowest prevalence rate (29%). The CUR population, as well as CANT, did not show beneficial effects, but a negative effect on fecundity and a neutral effect on the longevity of females, males and development time was observed. However, it presented a possible partial CI, which may explain a higher prevalence than the population of CANT, but still had the second lowest prevalence rate (40%).

The TIR population had a negative effect only on the longevity of females, in which the infected survived for less time than the uninfected. The beneficial effects of this population were observed for the longevity of infected males, which showed a longer lifespan than uninfected males, and for fecundity, a neutral effect was still observed for the development time, and the prevalence rate was intermediate at 50% (Table 3). The ELD and VIT populations were the only ones that did not present negative effects and were the ones with the highest prevalence rates, among those studied for these parameters, of 57% and 53%, respectively (Table 3). For ELD, a beneficial effect was observed for the longevity of both infected males and females that demonstrated a longer lifespan than the uninfected and a neutral effect for fecundity and development time. For VIT, a beneficial effect was observed for fecundity, in which infected females produced more than uninfected females, and neutral effects were observed for longevity and development time.

Table 3. Correlation between prevalence and effects on fitness. Red = negative effects; Green = beneficial effects; Gray = neutral effects.

	CANT	CUR	TIR	VIT	ELD
Fecundity					
Female longevity					
Male longevity					
Development time					
CI					
MK					
Prevalence	29%	40%	50%	53%	57%

4. DISCUSSION

The *wStv* strain was found to infect 14 populations of *D. sturtevantii*, with a mean prevalence of 41% and highly variable rates (0-70%), with no genetic variability observed from the 16S *rRNA* gene sequence. Likewise, the *wSuz* strain was also observed without genetic variability in 23 populations of *D. suzukii*, analyzed by MLST (Cattell et al., 2016). The high variation in the frequency of *Wolbachia* infection in natural populations of hosts can be explained by several factors, such as variable efficiency in transferring the strain from mother to offspring (vertical transmission), cost/benefits of infection for the population host, the genetic backgrounds of the host and the strain, and environmental factors such as temperature, humidity, diet, and others (Mouton et al., 2006; Yamada et al., 2007; Walker et al., 2011; Cattell et al., 2016). Based on the intermediate infection rate, we can suggest that this strain is not fixed in *D. sturtevantii* populations, which would be expected in cases of obligate mutualism, in which the host becomes dependent on *Wolbachia*, which promotes essential functions, such as reproduction and nutrition (Sicard et al., 2014; Nikoh et al. 2014). These intermediate rates may be associated with several factors, such as stochastic events, non-induction of CI or partial CI and imperfect maternal transfer (Giordano et al., 1995; Hoffmann et al., 1996; Charlat et al., 2004; Kriesner et al., 2013; Hamm et al., 2014; Cattell et al., 2016; Meany et al., 2019).

The effects of *Wolbachia* on the fitness of *D. sturtevantii*, analysed through reproductive parameters such as fecundity, longevity and development time, were varied, without a noticeable pattern. This variation may be related to the genetic background of the host population. The variation was evidenced for populations of *D. melanogaster*, in which infection with the *wMel* strain also showed variable results in relation to studies such

as sensitivity to cold and oxidative stress, fecundity and longevity. The explanation given by the authors was also that the effects of *Wolbachia* depend on the genetic background of the host (Serga et al., 2021). Furthermore, Strunov et al. (2022) also demonstrated with populations of *D. melanogaster* that the *Wolbachia*-host interaction depends on the environment (temperature factor) and on the genotype of both hosts and strains. Another interesting result was observed in the development time parameter. No direct effects of *Wolbachia* infection were observed for this parameter in *D. sturtevantii* populations, and this result corroborates Strunov et al. (2022) from studies with *D. melanogaster*. According to the authors, the lack of effect on development time may be related to the differential activity of *Wolbachia* in the early stages of host development, which may be causing a possible dormancy of the bacteria at this stage (Strunov et al., 2022).

An association of *Wolbachia* prevalence with effects on host fitness was also observed. The effects on fitness were positive/beneficial (7), neutral (18) and negative (5). Thus, populations with higher prevalence present more beneficial effects, and those with lower prevalence present more negative effects. We assume that with the increase in beneficial effects on the fitness of the host, *Wolbachia* infection will be able to maintain itself and spread in the host populations, as has already been observed with the wAu and wRi strains in *D. simulans* (Hoffmann and Turelli, 1997; Kriesner et al., 2013).

In the evaluation of reproductive manipulations, a female-biased sex ratio was observed in all types of crosses; however, only six crosses of the 15 analysed showed statistically significant differences, and all were related to infected females. Despite this, there is no way to associate these results with male killing (MK) manipulation, which, in addition to CI, is the only one that occurs in *Drosophila* species, since the sexual distortion caused by MK is strong, close to 100% of offspring composed of females, such as the wClay strain, infecting *Scaptodrosophila claytoni*, which over the generations showed 100% MK (Richardson et al., 2022); *D. bifasciata* (100%) (Hurst et al., 2000), *D. pandora* (98%) (Richardson et al., 2016) and *D. innubila* (97%) (Dyer and Jenike, 2004).

Cytoplasmic incompatibility (CI) was observed only as partially for the CUR and VIT populations and not in the other populations of the present work. The strain wStv has already been evaluated to induce strong CI in the transinfected host *D. simulans* (Martinez et al., 2015). This variation observed in the induction of CI can also be explained by the interaction of the strain with the genetic background of the host, as in the case of the wMel strain that causes CI in the transinfected host *D. simulans* and does not induce CI in *D. melanogaster*, its natural host (Poinsot et al., 1998; Gazla and Carrecedo, 2009). Thus,

the host background seems to have a strong influence on the effects produced by *Wolbachia* on their hosts and may be partly responsible for this range of variation observed for the fitness and CI results in the present work.

CI is associated with the rapid spread of *Wolbachia* in host populations, as it benefits infected females (Turelli et al., 1992). In host populations, the existence of *Wolbachia* strains that induce partial or no CI is frequent and present at low and intermediate frequencies of infection, such as the *wSuz* strain in *D. sukuzii* populations, which has an infection frequency of 17-46% (Hamm et al., 2014; Cattell et al., 2016), the *wAu* strain in *D. simulans* populations from Australia, with a 0-18% infection frequency (Hoffmann et al., 1996) and the strain *wMau* in *D. mauritiana*, with 34-46% (Giordano et al., 1995; Meany et al., 2019). The same was observed in the present study, in which it was possible to associate low and intermediate rates of prevalence with the noninduction of CI in the CANT, ELD, TIR populations and the partial induction of CI in CUR and VIT. Relatedly, a result that should be highlighted is the presence of genes responsible for inducing the manipulation CI (*cifA* and *cifB*) and a pair of pseudogenes in *wStv* (Martinez et al., 2021). The association between the presence of *cif* genes and the induction of CI is strong (Martinez et al., 2021). Thus, there is a possibility that the *wStv* strain induces CI, even if partial, in other populations of *D. sturtevantii*, which also presented an intermediate prevalence, in general.

Reproductive manipulation, such as CI, not only favours the spread of *Wolbachia* but also favours the spread of host mtDNA that is cotransmitted maternally together with *Wolbachia* by hitchhiking, resulting in linkage disequilibrium (Hurst and Jiggins, 2005). A question related to this topic was raised by Zorzato et al. (2022) regarding the interference of *Wolbachia* in the mtDNA variation of the same populations of *D. sturtevantii* evaluated in the present study. The authors, using the mitochondrial genes *COI* and *COII*, observed a high haplotype diversity and low nucleotide diversity. The question that remained open was whether the distribution and variation of haplotypes observed for populations were associated with geographic variables or whether there could be an influence of *Wolbachia* infection. The results of the present study suggest that there is no correlation with *Wolbachia* infection, as these populations presented only a single strain of *Wolbachia*, *wStv*, present in practically all populations (14), randomly distributed among populations with different mtDNA haplotypes, in variable prevalence rates and without genetic variability; therefore, it was not possible to correlate the haplotype diversity (mtDNA) found with the influence of the *wStv* strain.

The maintenance of the wStv strain in *D. sturtevantii* populations may be associated with the beneficial and/or neutral effects observed in the results of the evaluated reproductive parameters. The high variability in the response to the different parameters studied may be directly related to the genetic background of geographic populations, which present a level of genetic differentiation previously observed through microsatellite and mitochondrial markers (Trava et al., 2021; Zorzato et al. al., 2022). The genetic background of these populations is possibly responsible for the notorious association between the level of *Wolbachia* prevalence and the effect on fitness, where higher prevalence has higher fitness and lower prevalence has lower fitness.

In summary, the use of recently collected hosts is important to understand the complex interaction of *Wolbachia* infection and its hosts. Our unprecedented results for the neotropical species *D. sturtevantii*, with a wide geographic distribution and an evolutionary history still under construction, corroborated some of the results already obtained in the literature for other species of the genus, indicating that *Wolbachia modus operandi* may be variable in different related species. The understanding of the *Wolbachia modus operandi* is fundamental mainly because this bacterium has been used in the biological control of pests in agriculture (Bourtzis, 2008; Nikolouli et al., 2018; Mateos et al., 2020) and in the control of vector-borne diseases (Walker et al., 2011; Dutra et al., 2016; O'Neill et al., 2019).

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SUPPLEMENTARY MATERIAL

Supplementary Table S1. Primers used. T°C = annealing temperature.

Gene	Sense	Primers	Sequence of primers	T°C	Author
COI	Forward	TY-J-1460	5'-TACAATCTATCGCCTAAACTTCAGCC-3'	56°C	Simon et al., 1994
	Reverse	C-1-N-2191	5'-CCCGGTAAAATTTAAATATAAACTTC-3'	56°C	Sperling & Hickey, 1994
wsp	Forward	81F	5'-TGGTCCAATAAGTGATGAAGAAAC-3'	59°C	Zhou et al., 1998
	Reverse	691R	5'-AAAAATTAAACGCTACTCCA-3'	59°C	Zhou et al., 1998
16S rRNA	Forward	99F	5'-TTGTAGCCTGCTATGGTATAACT-3'	55°C	O'Neill et al., 1992
	Reverse	994R	5'-GAATAGGTATGATTTTCATGT-3'	55°C	O'Neill et al., 1992

Supplementary Table S2. Types of crosses and codes of the lines used in crosses. N = number of replicas used in each cross.

Population	Types of crosses	Crosses		N
	Female x Male	Female	Male	
Parque Estadual da Cantareira, SP, Brazil	UN x UN	CANT1	CANT1	3
	IN x IN	CANT3	CANT3	3
	IN x IN	CANT4	CANT4	3
	IN x IN	CANT5	CANT5	3
	UN x IN	CANT1	CANT3	3
	UN x IN	CANT1	CANT4	3
	UN x IN	CANT1	CANT5	3
	IN x UN	CANT3	CANT1	3
	IN x UN	CANT4	CANT1	3
	IN x UN	CANT5	CANT1	3
Floresta do Curió, Fortaleza, CE, Brazil	UN x UN	CUR16	CUR16	3
	IN x IN	CUR06	CUR06	3
	IN x IN	CUR08	CUR08	3
	IN x IN	CUR42	CUR42	3
	UN x IN	CUR16	CUR08	3
	UN x IN	CUR16	CUR42	3
	IN x UN	CUR08	CUR16	3
	IN x UN	CUR42	CUR16	3
Eldorado do Sul, RS, Brazil	UN x UN	ELD11	ELD11	3
	IN x IN	ELD06	ELD06	3
	IN x IN	ELD15	ELD15	3
	IN x IN	ELD21	ELD21	3
	UN x IN	ELD11	ELD15	3
	UN x IN	ELD11	ELD21	3
	IN x UN	ELD15	ELD11	3
	IN x UN	ELD21	ELD11	3
Reserva Biológica de Tirimbina, Sarapiquí, Costa Rica	UN x UN	TIR04	TIR04	3
	IN x IN	TIR10	TIR10	3
	IN x IN	TIR11	TIR11	3
	IN x IN	TIR12	TIR12	3
	UN x IN	TIR04	TIR10	3
	UN x IN	TIR04	TIR11	3
	UN x IN	TIR04	TIR12	3
	IN x UN	TIR10	TIR04	3
	IN x UN	TIR11	TIR04	3
	IN x UN	TIR12	TIR04	3
Parque Estadual da Fonte Grande, Vitoria, ES, Brazil	UN x UN	VIT05	VIT05	3
	IN x IN	VIT01	VIT01	3
	IN x IN	VIT06	VIT06	3
	IN x IN	VIT10	VIT10	3
	UN x IN	VIT05	VIT06	3
	UN x IN	VIT05	VIT10	3
	IN x UN	VIT06	VIT05	3
	IN x UN	VIT10	VIT05	3

Supplementary Table S3. Probability p values. Smaller than 0.05 ($p < 0.05$) show statistically significant difference (red). (Apêndice A)

4. CAPÍTULO II

High levels of horizontal transmission of *Wolbachia* in the genus *Drosophila*

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Abstract

Wolbachia is an endosymbiotic genus of Alphaproteobacteria that infects a wide range of arthropods and nematodes. This endosymbiont is typically transmitted maternally, from mother to offspring, but horizontal or non-maternal transmission between species has been reported. Horizontal transmission can occur by different routes in nature, through predation, parasitism, cannibalism, and shared ecological niches. This transmission is described as an infrequent event in the literature, and there are few well-reported studies on *Wolbachia* acquisition modes and divergence time. In the present work, we inferred the phylogenetic relationships and estimated divergence time of 67 *Wolbachia* strains from supergroup A, infecting 54 hosts, in order to evaluate the mode of acquisition of the supergroup A strains that infect the *Drosophila* genus. The results indicated a high frequency of horizontal transmissions and the occurrence of at least eight independent invasions of *Wolbachia* strains in the genus *Drosophila*, which continued to spread among the species of the innermost clades, by other horizontal transmissions over evolutionary time. It was also observed that the infecting strains of sister host species were acquired via horizontal transmission and not by cladogenic or introgression, as is expected in theory.

Keywords: *Drosophila* species, host shifts, transmission routes, strains, supergroup A.

Introduction

Wolbachia is an endosymbiont belonging to the order Rickettsiales (Hertig and Wolbach 1924) that infects both arthropods and nematodes (Zug and Hammerstein 2012; Lefoulon et al. 2016; Sazama et al. 2017). *Wolbachia* is often found reproductively manipulating its hosts, consequently increasing its spread in host populations (Yen and Barr 1971; Rousset et al. 1992; O'Neill et al. 1998; Kriesner et al. 2016). Transmission is one of the main processes closely related to successful endosymbiont establishment in host populations. It is known that the main means of transference of *Wolbachia* is maternally, from mother to offspring, called vertical transmission from the cytoplasm of eggs (Werren 1997), but horizontal transmissions have already been recorded (O'Neill et al. 1992; Wolfe et al. 2021; Gomes et al. 2022). The modes and divergence times in

which strains are acquired by hosts are still poorly studied (O'Neill et al. 1992; Rousset and Solignac 1995; Huigens et al. 2004; Turelli et al. 2018; Cooper et al. 2019).

The acquisition of *Wolbachia* by the host can occur in three ways: by cladogenic acquisition, in which sister species inherited the *Wolbachia* strain from the recent ancestor during speciation (Raychoudhury et al. 2009; Gerth and Bleidorn 2016); introgression and hybridization, in which phylogenetically close species hybridize, acquiring the strain of the sister species (Rousset and Solignac 1995; Raychoudhury et al. 2009), as is the common case in species of the genus *Drosophila* (Coyne and Orr, 1989; 1997; Garrigan et al. 2012; Turelli et al. 2014; Cooper et al. 2019); and horizontal acquisition (O'Neill et al. 1992; Turelli et al. 2018) in which the strain is transferred non-maternally, either by direct or indirect interaction of phylogenetically close or distant hosts (O'Neill et al. 1992; Baldo et al. 2008; Gerth and Bleidorn 2016; Turelli et al. 2018). However, in horizontal acquisition it has been observed that some closely related strains infect phylogenetically close hosts, which have a similar genetic background, suggesting that there is some degree of specificity of these strains (Baldo et al. 2008).

The fact that *Wolbachia* can survive in the extracellular environment for long periods, despite being an obligatory intracellular endosymbiont, and its ease in recombining and adapting to different genomes and environments, ease in moving through tissues and cells, contribute to the non-maternal transfer of that organism (Tolley et al. 2019; Sanaei et al. 2021; Strunov et al. 2022). It has already been observed that horizontal transfer occurs regardless of physical cell-to-cell contact, which *Wolbachia* manages to survive in the culture medium, with transfer also occurring from the culture medium to the cell (White et al. 2017). The routes of horizontal transmission can be diverse, such as through parasitism (Brown and Lloud 2015), cannibalism (Su et al. 2019), predation (Le Clec'h et al. 2013) and through the sharing of ecological niches (Li et al. 2017; Su et al. 2019). Among *Drosophila* species, it has already been discussed that horizontal transmission can occur through parasitoid wasps (Vavre et al. 1999), mites (Brown and Lloud 2015), shared niches (Mateos et al. 2006) and even cannibalism between larvae (Haine et al. 2005).

It is possible to study how strains are acquired by comparing phylogenies and estimating the divergence time between *Wolbachia* and host genomes, both mitochondrial DNA (mtDNA) and nuclear DNA (nDNA) (Raychoudhury et al. 2009; Conner et al. 2017; Turelli et al. 2018). The agreement between the phylogenies and estimates of divergence time among the three genomes (*Wolbachia*, nDNA and mtDNA), cladogenic acquisition is

suggested (Conner et al. 2017; Turelli et al. 2018; Cooper et al. 2019). The agreement only between the phylogenies and estimates of divergence time among *Wolbachia* and mtDNA, it supports introgressive acquisition (Conner et al. 2017; Turelli et al. 2018; Cooper et al. 2019). Finally, no agreement between any phylogeny and estimated divergence time, it is suggested that *Wolbachia* was acquired horizontally (Conner et al. 2017; Turelli et al. 2018; Cooper et al. 2019).

In the literature, horizontal transmission is reported as infrequent and the scarcity of well-documented studies with reliable markers in relation to the mode of acquisition and divergence time estimation may contribute to this fact found in the literature (Huigens et al. 2004; Hamm et al. 2014; Turelli et al. 2018; Wolfe et al. 2021). A study on the effects of *wStv* strain infection on *D. sturtevantii* host populations (Roman et al. 2022, submitted to the Journal of Evolutionary Biology) and the observation of horizontal acquisition of this strain (Miller and Riegler 2006), motivated us to analyze the phylogenies and divergence times of the supergroup A *Wolbachia* strains, to which *wStv* and most of the infecting strains of *Drosophila* are inserted, as well as their hosts, in order to evaluate the mode of acquisition of *Wolbachia*. Focusing on the genus *Drosophila*, a high frequency of horizontal transmission was observed, with at least eight recent invasions throughout the evolutionary history of this genus. Among these observed horizontal transmissions, we highlight the close relationship of *wStv* and *wLeh* strains with ant infecting strains, such as *wAdent* and *wAgra*, never described before.

Materials and Methods

Genome assembly

The genomes of *D. sturtevantii*, *D. lehrmanae*, *D. septentrionalis*, *D. pseudosaltans* and *D. prosaltans* were sequenced in pools of individuals by Prediger et al., 2022 (in preparation). From these genomes, the reads were mapped on *Wolbachia pipientis* strain infecting *D. sturtevantii* (*wStv*; ASM1410747v1; Martinez et al. 2021), using Minimap2 program package (Li 2018). Minimap2-generated SAM files were converted to BAM format using samtools 1.9 software (Li et al. 2009), cleaned and sorted using Picard v.2.0.1. and then converted to Fasta using samtools' subpackage bcftools, which generated for each species a pseudo-reference genome by replacing sites in the *wStv* genome by the strain variants. These pseudogenomes were then annotated, identified by BLAST and used in the two items described below.

Phylogenomics analysis

In the present work, phylogenies and divergence time estimates were performed only with the genomes of *Wolbachia* strains and mitochondrial genomes of the hosts, in order to observe possible horizontal transmission. Nuclear genomes were not used because for many hosts data were not found. To generate the phylogenetic tree of *Wolbachia*, in addition to the seven genomes sequenced by us, we performed a wide sampling search of supergroup A strains in the GenBank database, totaling 67 strains from supergroup A and four from supergroup B (outgroup) (Supplementary Table S1). The tree was built using single-copy orthologous (SCO), which were identified in the OrthoFinder program (Emms and Kelly 2015). The total SCO found was 122 genes, which were aligned in MAFFT (Kato 2013) and trimmed in TrimAl (Capella-Gutierrez et al. 2009), using the standard configuration. Evolutionary relationships were inferred by maximum likelihood (ML) in the IQ-Tree program (Nguyen et al. 2015). Branch support was estimated with ultrafast bootstrap (UFBoot2) (Hoang et al. 2018), using 10,000 replicates. For the phylogenetic trees of the hosts, 13 mitochondrial genes from 54 species were used (Supplementary Table S2). Phylogenetic relationships were inferred by ML in the IQ-Tree program as described above (Nguyen et al. 2015).

P-distance and divergence time estimation

P-distances were estimated for *Wolbachia* strains based on third-position substitution rate of the 122 SCOs (40,376 bp analyzed). For hosts, the substitution rate of all three codon positions of the 13 mitochondrial genes were considered, totaling 11,027 bp analyzed. These analyzes were performed using the MEGA program (Kumar et al. 2018).

To estimate the divergence time, we consider that the pairwise genetic distance is directly related to the divergence time (Nei 1972). In this way, we calculate the divergence times from the genetic distances, using the formula below: where “t” is the divergence time, “D” the genetic distance and “u” the mutation rate. The mutation rates used for the divergence times estimation were $u = 6.87 \times 10^{-9}$ at third-position site per year for *Wolbachia* (Richardson et al., 2012) and $u = 6.2 \times 10^{-8}$ for all three codon positions sites per year for hosts (Haag-Liautard et al. 2008).

$$t = \frac{D}{2u} (1)$$

Results

The strains found in the *D. sturtevantii*, *D. lehrmanae*, *D. prosaltans* and *D. septentriosaltans* genomes were respectively: wStv, wLeh, wPro and wSpt, previously described (Miller and Riegler 2006; Strunov et al. 2022); and the wPsd strain was found in the genome of *D. pseudosaltans*, observed for the first time in the present work. These strains, as well as the host genomes, were used in phylogenetic inference with the other sequences taken from the Genbank database. The cophylogeny (*Wolbachia* and their hosts) are shown in fig. 1 and phylogenetic trees of the strains and hosts are respectively in the supplementary fig. S1 and S2.

In the present phylogenetic analysis of the strains, supergroup A was composed of 67 strains, of which 45 infect 27 species of *Drosophila* and 22 strains infect the other host species belonging to the orders Diptera, Hemiptera, Hymenoptera and Lepidoptera (Supplementary Table S1). These supergroup A strains clustered in the phylogenetic tree, generally incongruously with the tree of their hosts (Fig. 1). The wStv and wLeh strains infecting the sister species *D. sturtevantii* and *D. lehrmanae*, respectively, clustered in the most divergent clade of supergroup A, with high support (100) (Fig. 1). It was observed that wStv had the lowest genetic distance and the shortest divergence time with the wLeh strain (~46,000) (Fig. 2; Supplementary Table S3 and S4). The divergence time was different when compared between the hosts, based on mitochondrial genes, *D. sturtevantii* and *D. lehrmanae* (~1.9 mya) (Supplementary Table S5), in which they were considerably longer than estimated for the strains. These host species are sisters and the acquisition of *Wolbachia* could have occurred by cladogenic or introgressive way, however, these results show us that the acquisition is more recent than the divergence of hosts and hybridization cases, being supported here the form of acquisition horizontal for both hosts.

The wStv and wLeh strains were positioned phylogenetically distant from the large clade composed of the infecting *Drosophila* strains, as well as from the infecting strains of phylogenetically correlated species such as wPro (*D. prosaltans*), wSpt (*D. septentriosaltans*) and wPsd (*D. pseudosaltans*), the genetic distance of ~0.03 and divergence time of ~2.5mya between the wStv-wLeh and wPro-wSpt-wPsd clades (Figs. 1 and 2; Supplementary Table S3 and S4). And both (wStv and wLeh) positioned

themselves in a clade shared by the following strains: *wAdent* (*Apterostigma dentigerum*), a fungus-growing ant, with a genetic distance of 0.008 and a divergence time of ~605,000 years; *wAgra* (*Anoplolepis gracilipes*), known as yellow crazy ant, with genetic distance of 0.01 and divergence time of ~1.3mya; and the *wNik* strain that infects a species of the *Drosophila melanogaster* group (*D. nikananu*), with genetic distance of 0.02 and divergence time of ~1.5mya (Figs. 1 and 2; Supplementary Table S3 and S4). The incongruities between the phylogenies and the estimated divergence times of the strains and hosts (based on mitochondrial genes) strongly support the horizontal transmission mode within this clade (*wStv-wLeh- wAdent- wAgra- wNik*) (Figs. 1 and 2; Supplementary Table S3 and S5).

In the remainder of the analysis on the phylogenetic tree of *Wolbachia*, it was observed that most of the infecting strains of *Drosophila* were grouped into two major clades (Fig. 1). One of them is composed of the strains *wRi* (*D. simulans*), *wAna* (*D. ananassae*), *wSuz* (*D. suzukii*), *wSpc* (*D. subpulchrella*), *wTri* (*D. triauraria*) and *wAur* (*D. auraria*). This clade represents one of the most recent in the analyzed phylogenetic tree, with an estimated divergence time between 3,614 and 18,000 years, which when compared to the divergence of their hosts, based on mitochondrial genes, an incongruity is observed, with divergence of at least 800,000 years to 8 mya, based on the mitochondrial genes (Fig. 2; Supplementary Table S3 and S5). Thus, it is possible to infer that *Wolbachia* was recently acquired by these *Drosophila* species. And analyzing the internal clades, we were able to infer other specific horizontal transmissions, among the most related strains, such as: *wTri* and *wAur*, infecting the sister species *D. triauraria* and *D. auraria*, respectively; *wSuz* and *wSpc*, whose host species are closely related also *D. suzukii* and *D. subpulchrella*, respectively; and *wRi* and *wAna*, infecting species of the *melanogaster* group *D. simulans* and *D. ananassae*, respectively (Figs. 1 and 2; Supplementary Table S3 and S6).

The largest clade of infective strains of the genus *Drosophila* is composed of 22 infective strains of 9 species of the subgenus *Sophophora*, four of the subgenus *Drosophila* and one of the genus *Rhagoletis*: *wPro*, *wPsd*, *wSpt*, *wTro*, *wAu*, *wTei*, *wYak*, *wSan*, *wMel*, *wRec*, *wAra*, *wInc*, *wBor* and *wCin2* (Fig. 1). These strains diverged within this clade between 3,615 and 170,000 years ago (Supplementary Table S3). It was possible to observe in the internal clades the phylogenetic proximity between the strains that infect the *saltans* group of *Drosophila*, such as *wPro* (*D. prosaltans*), *wPsd* (*D. pseudosaltans*) and *wSpt* (*D. septentrionsaltans*) (Fig. 1). Considering that their hosts are

from the same phylogenetic subgroup (*saltans*) and the short genetic distance and divergence time (62,000 to 77,000 years) among these strains, we could expect a cladogenic or introgressive transmission, however their hosts diverged ~2 mya ago (based on mitochondrial genes), which suggests a more recent acquisition, via horizontal transmission (Figs. 1 and 2; Supplementary Table S3 and S6).

Furthermore, the relationship of the *wTei*, *wYak* and *wSan* strains was observed, which are infective of the sister species, *D. teissieri*, *D. yakuba* and *D. santomea* (Fig.1). These three strains were recently acquired ~3,615 years ago, disagreeing with the hosts' divergence time of 130,000 to 300,000 years (based on mitochondrial genes), which rules out acquisition via introgression, demonstrating the most recent acquisition, horizontal transmission (Fig. 2; Supplementary Table S3 and S6). It was also observed the proximity between the *wTro* and *wAu* strains, which infect the hosts *D. tropicalis* (*willistoni* group of *Drosophila*) and *D. simulans* (*melanogaster* group of *Drosophila*), respectively, which are phylogenetically more distant and have a longer divergence time, also suggesting horizontal transmission (Figs. 1 and 2; Supplementary Table S3 and S6).

In the large clade, the *wMel* strain and its variants, infecting *D. melanogaster*, as well as the infecting strains of species of the *Drosophila* subgenus, *wRec* (*D. recens*), *wAra* (*D. arawakana*), *wInc* (*D. incompta*), *wBor* (*D. borealis*), and a fly of the genus *Rhagoletis* *wCin2* (*Rhagoletis cingulata*), did not form internal clades, they were just close to each other and with the other clades already mentioned (Fig.1). Although these strains show short genetic distance between them, their hosts are not phylogenetically close, indicating horizontal transmission (Figs. 1 and 2; Supplementary Table S4).

In addition to these clades already mentioned, it is also possible to observe other infecting strains of the *Drosophila* genus dispersed in other parts of the phylogenetic tree closely related to other infecting strains of Diptera, which by the phylogeny of the hosts, it is possible to suggest horizontal transmission: such as *wBic* (*D. bicornuta*) related to *wCer1* (*Rhagoletis cerasi*); and *wBif* (*D. bifasciata*) with *wGmm* (*Glossina morsitans*) (Figs. 1 and 2; Supplementary Table S3 and S4). There are also inconsistencies in the relationship between *wHa* and *wSh* strains, which infect sister *Drosophila* species, *D. simulans* and *D. sechellia*, with *wCobs*, *wKgib* and *wCsol* strains, which infect Hymenoptera species (*Cardiocondyla obscurior*, *Kradibia gibbosae* and *Ceratosolen solmsi*, respectively) and the *wCauA* strain that infects a Lepidoptera species (*Carposina sasakii*), suggesting horizontal transmission (Figs 1 and 2). Acquisition via introgression or cladogenic could be suggested between *wHa* and *wSh*, however, again it is possible to

observe that the divergence time between the strains is more recent than that of the hosts (based on mitochondrial genes), leading us to consider horizontal transmission (Fig. 1; Supplementary Table S3 and S5). Another case of introgression could be suggested between the wOrie and wNeo strains, which are closely related and with a recent divergence time, but their hosts, which are also closely related, *D. neotestacea* and *D. orientacea*, respectively, have a longer divergence time, suggesting horizontal transmission (Figs. 1 and 2; Supplementary Table S3 and S6). The wBai strain (*D. baimaii*) is not closely related to any other strain of the phylogenetic tree, showing divergence time between 1.3 to 3.0 mya with the others (Fig. 1; Supplementary Table S3).

Fig. 1. Cophylogeny of *Wolbachia* supergroup A (right) and their hosts (left). Branches with support above 95% probability are shown.

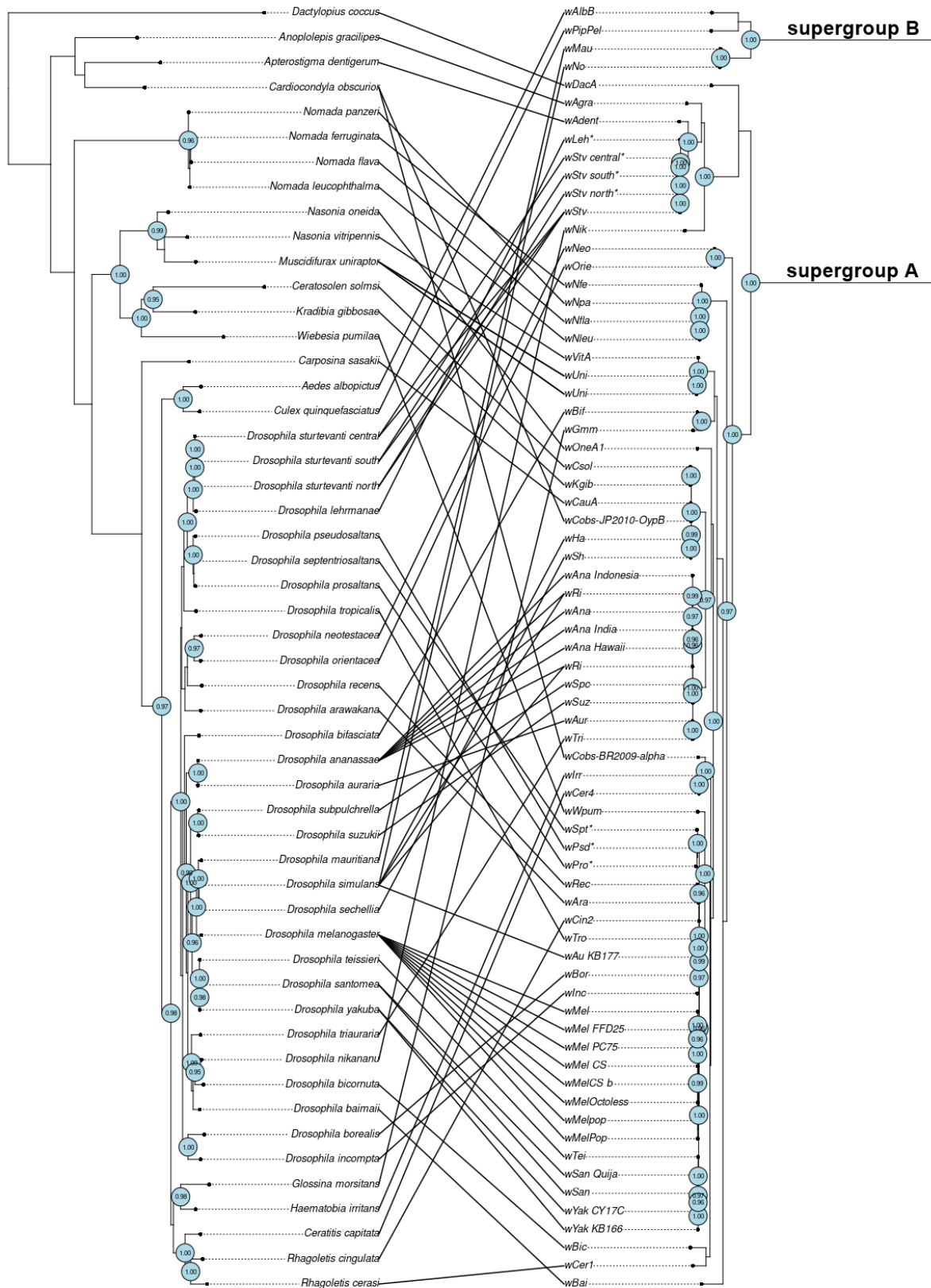
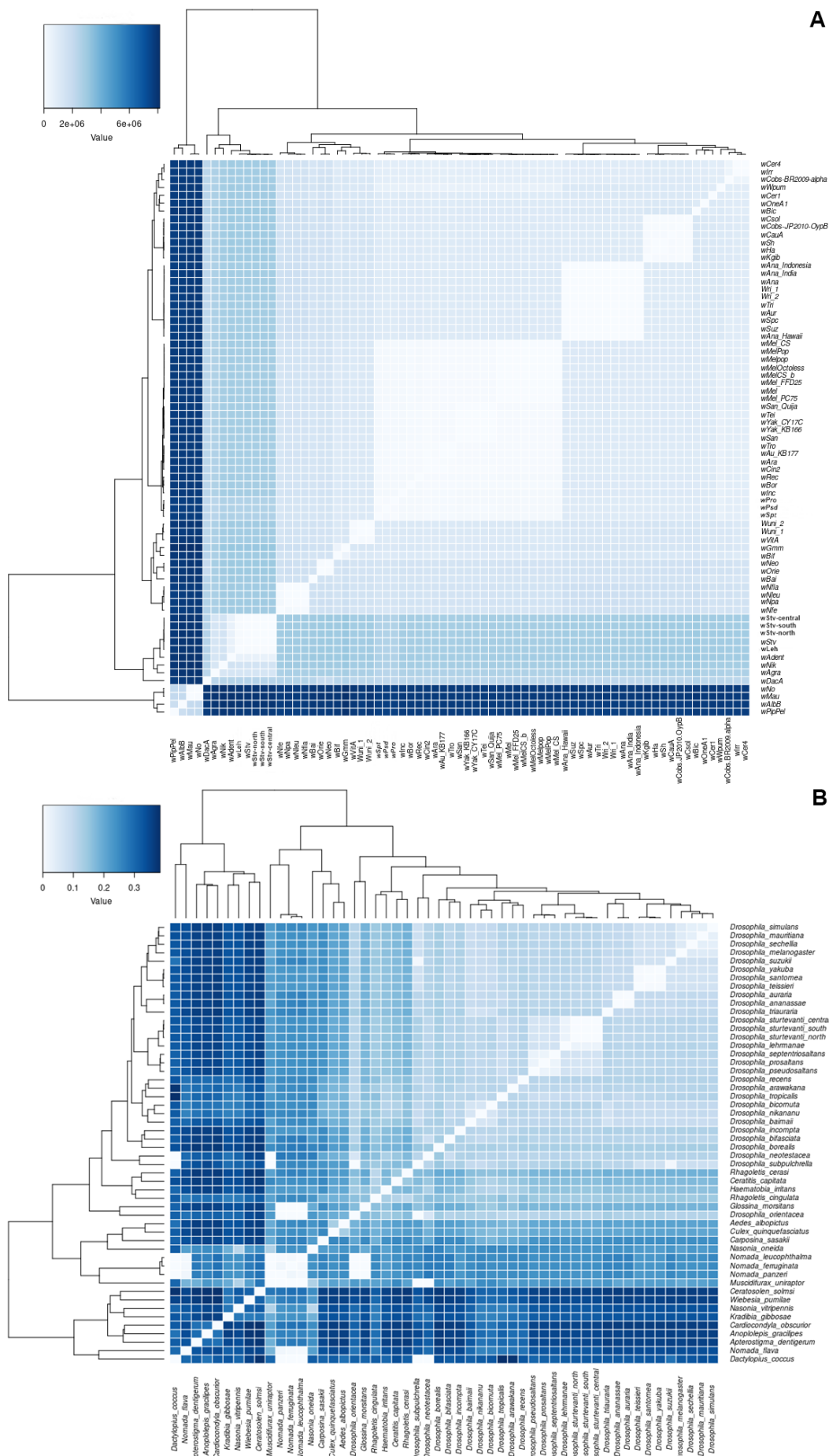


Fig. 2. Heatmap of the pairwise genetic distance of *Wolbachia* strains of the supergroup A (A) and their hosts (B). The clusters were identified through hierarchical analysis. The intensity of the staining is proportional to the genetic distance, the darker the greater the distance.



Discussion

In all clades composed of infecting *Drosophila* strains, it was possible to observe phylogenetic and divergence time incongruities between strains and hosts, demonstrating a high frequency of horizontal acquisitions. The results indicate that there were at least eight independent invasions of *Wolbachia* strains in the genus *Drosophila* and that after these events, the strains continued to spread through other horizontal transmissions. In the literature, horizontal transmission is often reported as an uncommon event, however, the results presented here indicate that it is more frequent than has been reported (Huigens et al. 2004; Hamm et al. 2014; Turelli et al. 2018). The rare occurrence of this transmission in the literature can be explained by the few studies related to the mode of acquisition and divergence time estimation, studies focused on small clades with low sample numbers, which often do not show the occurrence of horizontal transmission (Conner et al. 2017; Turelli et al. 2018; Cooper et al. 2019). The lack of use more reliable markers, such as genomic data, also makes it difficult to observe this mode of transmission, for example, Wolfe et al. (2021) using genomic data, were able to differentiate the *wCin* and *wCer* strains, infecting flies of the genus *Ragholetis* (*R. cingulata* and *R. cerasi*, respectively), not differentiated before by the MLST methodology, and suggested possible occurrences of transmission horizontally in these species.

Other studies, as well as ours, also observed a high frequency of horizontal transmission (Haine et al. 2005; Baldo et al. 2006; Wallau et al. 2016; Gomes et al. 2022). Baldo et al. (2006), already discussed that horizontal transmissions within the genus *Drosophila* are frequent, due to the similarity of the infecting strains of phylogenetically distant host species. Gomes et al. (2022) observed that several phylogenetically distant arthropod host species presented strains with high genetic similarity, of which of 17 analyzed host species, at least 10 shared *Wolbachia* strains, demonstrating that horizontal transmission is frequent. Likewise, Wallau et al. (2016), through the phylogeny of the MLST and *wsp* genes, also observed several horizontal transmission events within the supergroup A.

Some strains evaluated here have already been studied in previous works. The phylogenetic relationship between the *wTri*, *wAur*, *wSpc*, *wSuz*, *wRi* and *wAna* strains, considered to be *wRi*-like variants, has already been evaluated and it was observed that they diverged ~14,000 (5,000 - 27,000 years ago), very similar to what we found in present work, divergence of 3,614 to 18,000 years (Turelli et al. 2018). As in our work, horizontal

acquisition of *w*Ri and *w*Ana strains by hosts *D. simulans* and *D. ananassae* was observed, possibly through an intermediate host (Turelli et al. 2018). The relationships between the *w*Suz and *w*Spc strains, and between *w*Tri and *w*Aur, have also been discussed and because their hosts are closely related sister species (*D. suzukii* and *D. subpulchrella*; *D. triauraria* and *D. auraria*) it was not possible to distinguish the form of acquisition between introgression and horizontal transmission (Conner et al. 2017; Turelli et al. 2018), contrary to our results that showed only horizontal transmission.

Opposite to what we observed, it was verified the probable introgressive acquisition of *Wolbachia* within the *yakuba* clade, which comprises the species *D. yakuba*, *D. santomea* and *D. teisseieri*, infected by the *w*Yak, *w*San and *w*Tei strains, respectively (Cooper et al. 2019). In that same work, it was estimated that *w*Yak, *w*San and *w*Tei diverged from *w*Mel (*D. melanogaster*) ~29,000 years ago (Cooper et al. 2019), in disagreement with our work ~76,000 years ago. As in the present work, horizontal transmission has also been observed between the species *D. recens* and *D. melanogaster*, infected by the *w*Rec and *w*Mel strains, respectively (Metcalf et al. 2014). According to Metcalf et al. (2014), it is likely that some parasitoid wasp was the vector for horizontal transmission to occur between both host species, as there was no geographic overlap between them. And between *w*Ha and *w*Sh strains, contrary to what we found, it was suggested that they spread via introgression, due to the high similarity of the strains and their hosts *D. simulans* and *D. sechellia* (Hague et al. 2020).

The close relationship of the *w*Stv and *w*Leh strains that infect the sister species *D. sturtevantii* and *D. lerhmanae*, with the *w*Adent and *w*Agra strains infecting the two ant species *Apterostigma dentigerum* and *Anoplolepis gracilipes* and with the *w*Nik strain, infecting the species *D. nikananu* (*Drosophila melanogaster* group), was observed for the first time in the present work. Previously, it has been observed that the *w*Stv strain is phylogenetically distant from the other infecting strains of *Drosophila*, as well as *w*Nik (Mateos et al. 2006; Miller and Riegler 2006; Martinez et al. 2014; 2015; Strunov et al. 2022). Furthermore, recently from the results of a study of *Wolbachia* tropism in soma and germ line, a hypothesis was raised that the common ancestor of *D. sturtevantii* and *D. lerhmanae* carried a *w*Au-like strain, of which many *Drosophila* species, including those of the *saltans* group (*w*Pro, *w*Psd and *w*Spt) carry, and that this strain was replaced by a *w*Stv-like strain in an evolutionarily recent period (Strunov et al. 2022). This study suggests that the acquisition of the *w*Stv strain was cladogenic, that a common ancestor acquired this strain and it diverged together with the host species *D. sturtevantii* and *D. lerhmanae*.

(Strunov et al. 2022), which does not corroborate with our findings, on the horizontal acquisition.

In the present work, as observed, many horizontal transmissions occurred between phylogenetically close host species, where it would expect to find cladogenic or, mainly introgressive, transmissions, since hybridizations are common in species of the genus *Drosophila* (Turelli et al. 2014; Cooper et al. 2019). A possible explanation for these events is related to the fact that the interaction between the genetic background of the *Wolbachia* and its host is an important factor in reproductive manipulation and in the effect of fitness (Poinsot et al. 1998; Gazla and Carrecedo 2009; Serga et al. 2021; Strunov et al. 2022; Roman et al. submitted to Journal of Evolutionary Biology), thus, genetically similar hosts are likely to respond to bacteria more similarly. These results suggest that there may be a greater chance of *Wolbachia* transfer between closely related hosts, due to a possible specialization of strains in these hosts (Jiggins et al. 2002; Baldo et al. 2008), not excluding the transfer of strains between distant phylogenetics hosts, as is also observed in the present phylogenetic tree. This pattern of transfer of strains between phylogenetically close hosts has also been observed within the genus *Agelenopsis* (Baldo et al. 2008).

For the understanding of non-maternal transmission by hosts, ecological routes are an important issue. The horizontal transmission routes for each case observed here were not directly investigated, requiring studies in the ecological context. However, some routes have already been elucidated, showing how some of these horizontal transmissions can occur. The routes of this mode of transmission can occur via a vector or directly, via exposure to the external environment (Heath et al. 1999; Cordaux et al. 2001; Baldo et al. 2008; Ahmed et al. 2015; Li et al. 2017; Sanaei et al. 2021). Transmission via parasitoids has already been observed in *Drosophila* species, which found identical (or similar) *Wolbachia* strains between *Drosophila* and parasitoid wasps (Hymenoptera) (Vavre et al. 1999). This route is often speculated in works on horizontal transmission between *Drosophila* species (Miller and Riegler 2006; Metcalf et al. 2014).

Mites, which are ectoparasites of *Drosophila*, are also possible vectors in the transmission of *Wolbachia* between *Drosophila* species. *Wolbachia* transmission via the interaction of the *Tyrophagus putrescentiae* (white mite) and *D. melanogaster* species has already been observed in laboratory stocks (Brown and Lloyd 2015). Shared niches, such as food sources, can be great environments for *Wolbachia* propagation, as in the case of *Drosophila* species, its immature stages are saprophytes and feed on yeasts on

decomposing plant material, and this environment can be a transmission route for *Drosophila* species as well (Throckmorton 1975; Mateos et al. 2006).

The transmission routes of the clade of *wLeh*, *wStv* and *wAdent*, for the first time seen here, are speculative. Focusing on sister species *D. sturtevantii* and *D. lerhmanae*, the route may have occurred through the sharing of food sources, oviposition sources and parasitoid wasps, as these species were already found in sympatry, as in places in French Guiana (Madi-Ravazzi et al. 2021; Strunov et al. 2022). Furthermore, cannibalism among *Drosophila* larvae has been discussed as a possible route of transmission as well (Haine et al. 2005). The relationship of the *wStv* and *wLeh* strains with *wAdent*, which infects a fungus-growing ant, *Apterostigma dentigerum*, is an open question, even though the host species co-occur in Central and South America, their ecological interaction is unknown. Therefore, we suggest the existence of intermediate hosts to these, which have not yet been sequenced.

In summary, due to the high similarity of the infecting strains from phylogenetically distant hosts, as well as the recent divergence time of the strains compared to the divergence time of the hosts, a high frequency of horizontal transmission was shown in the species of the genus *Drosophila*. Thus, the results presented here clearly demonstrate that horizontal transmission is an important means of dispersion of *Wolbachia* in hosts of this genus. But some questions still remain open regarding the routes of these transmissions. There is a probability of horizontal transmission occurring in the direct via, due to the geographical overlap of some host species and the sharing of niches; and in the case of non-overlapping of niches, transmissions can be explained by the existence of intermediate hosts and missing links. The expansion of ecological studies among *Wolbachia* hosts and the sequencing of genomes on a large scale should be crucial points to clarify the possibilities of transmission routes.

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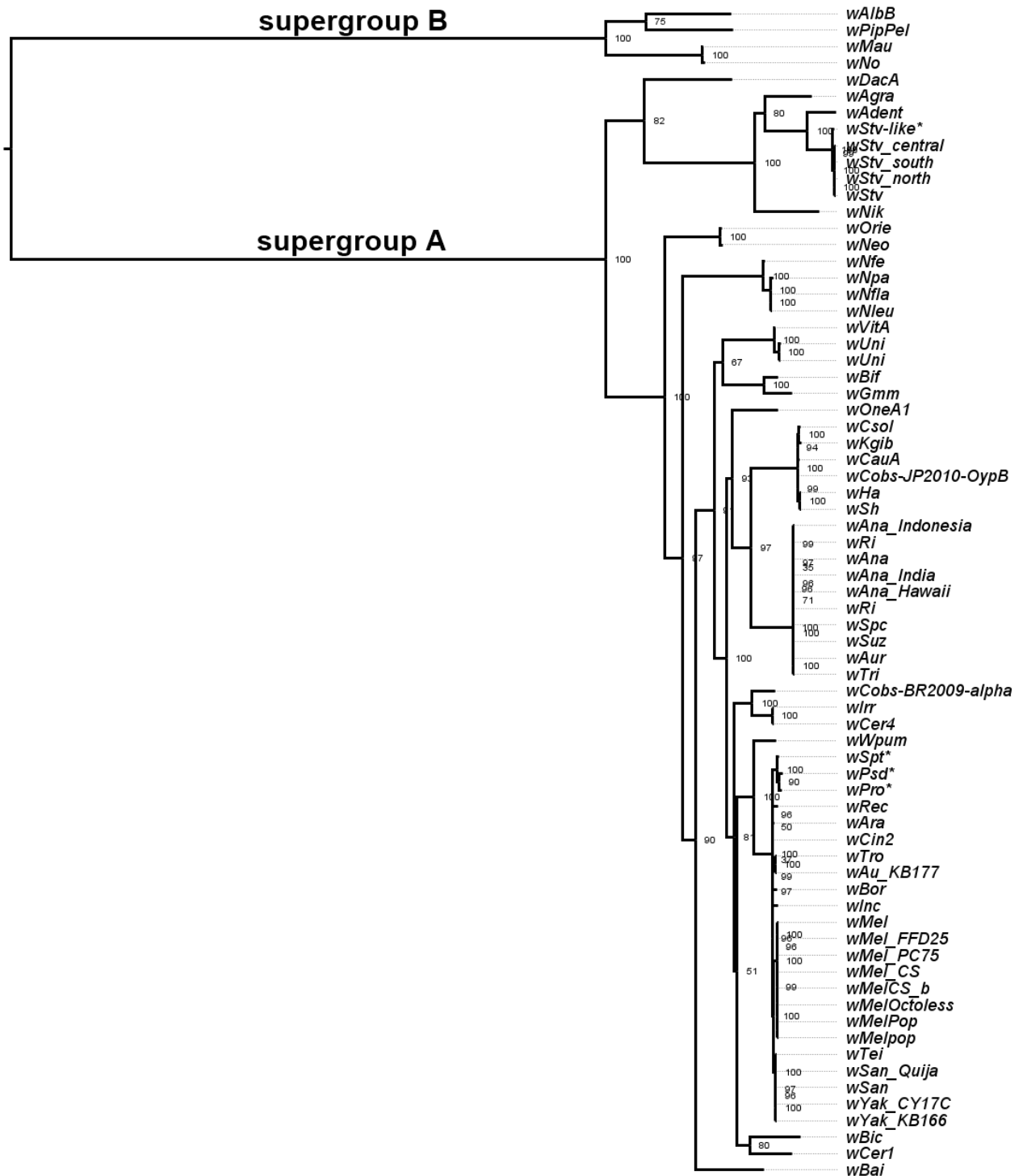
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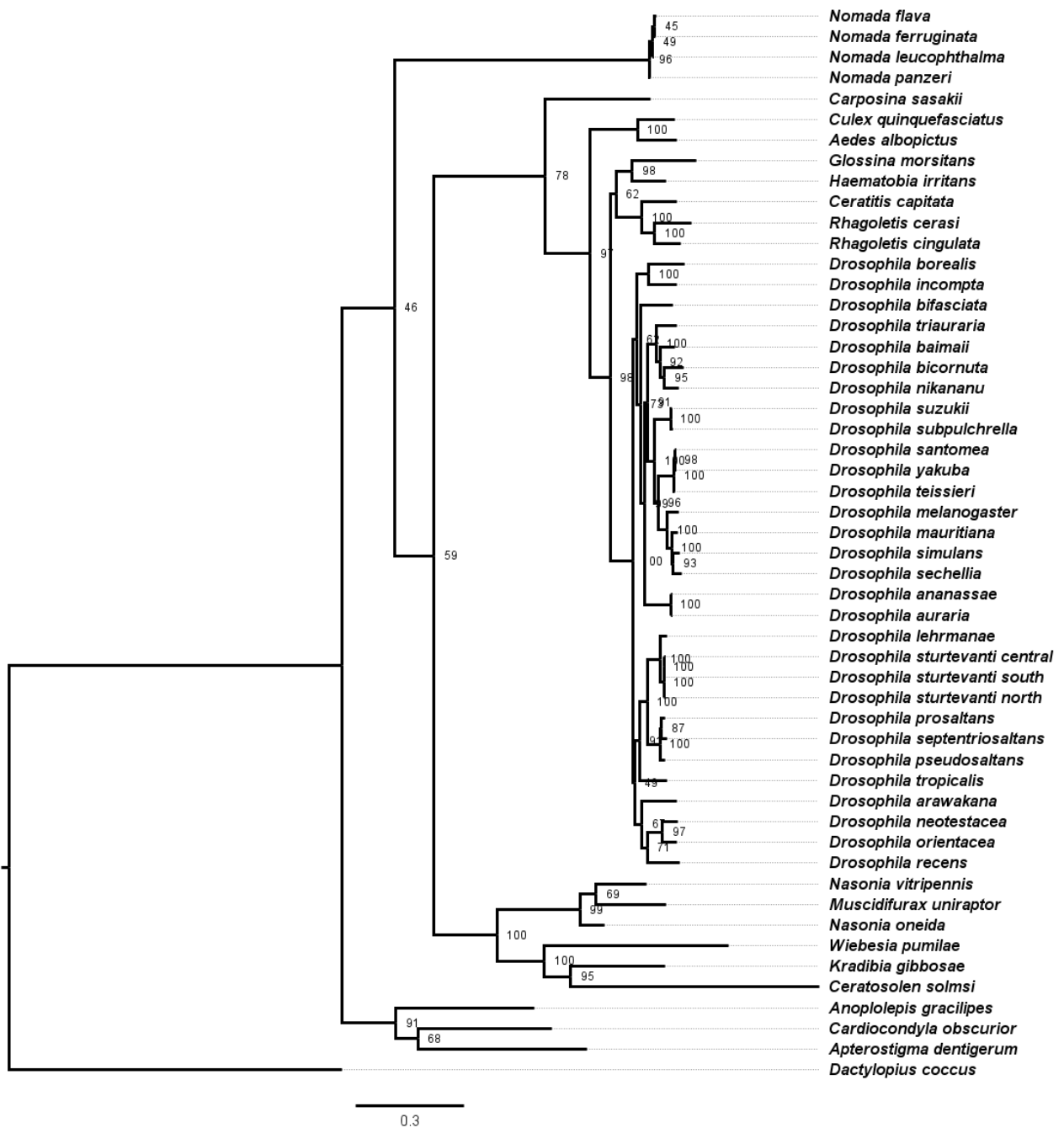
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SUPPLEMENTARY MATERIAL

Supplementary Figure S1. Phylogeny of supergroup A *Wolbachia* strains. Supergroup B as outgroup. The branches show support values.



Supplementary Figure S2. Phylogeny of hosts. *Dactylopius coccus* (Hemiptera) as outgroup. The branches show support values.



Supplementary Table S1. *Wolbachia* strains Information and its GenBank accession number.

<i>Wolbachia</i> strain	<i>Wolbachia</i> supergroup	Host Order	Host species	Accession number
wCer4	A	Diptera	<i>Ceratitis capitata</i>	GCF_018454455.1
wRi	A	Diptera	<i>Drosophila ananassae</i>	GCF_002907405.1
wAna_India	A	Diptera	<i>Drosophila ananassae</i>	GCF_003671365.1
wAna_Indonesia	A	Diptera	<i>Drosophila ananassae</i>	GCF_003671375.1
wAna_Hawaii	A	Diptera	<i>Drosophila ananassae</i>	GCF_003671405.1
wAna	A	Diptera	<i>Drosophila ananassae</i>	GCF_008033215.1
wAra	A	Diptera	<i>Drosophila arawakana</i>	GCF_014129655.1
wAur	A	Diptera	<i>Drosophila auraria</i>	GCF_017916175.1
wBai	A	Diptera	<i>Drosophila baimaii</i>	GCF_014129605.1
wBic	A	Diptera	<i>Drosophila bicornuta</i>	GCF_014129645.1

wBif	A	Diptera	<i>Drosophila bifasciata</i>	GCF_014129685.1
wBor	A	Diptera	<i>Drosophila borealis</i>	GCF_014129615.1
wInc	A	Diptera	<i>Drosophila incompta</i>	GCF_001758565.1
wLeh*	A	Diptera	<i>Drosophila lehrmanae</i>	Present work
wMel_CS	A	Diptera	<i>Drosophila melanogaster</i>	GCF_014354335.1
wMel_PC75	A	Diptera	<i>Drosophila melanogaster</i>	GCF_014354345.1
wMelpop	A	Diptera	<i>Drosophila melanogaster</i>	GCF_016584325.1
wMelOctoless	A	Diptera	<i>Drosophila melanogaster</i>	GCF_016584375.1
wMelCS_b	A	Diptera	<i>Drosophila melanogaster</i>	GCF_016584405.1
wMel	A	Diptera	<i>Drosophila melanogaster</i>	GCF_016584425.1
wMel_FFD25	A	Diptera	<i>Drosophila melanogaster</i>	GCF_017916155.1
wMelPop	A	Diptera	<i>Drosophila melanogaster</i>	GCF_000475015.1
wNeo	A	Diptera	<i>Drosophila neotestacea</i>	GCF_014129535.1
wNik	A	Diptera	<i>Drosophila nikananu</i>	GCF_014107455.1
wOrie	A	Diptera	<i>Drosophila orientacea</i>	GCF_014129565.1
wPro*	A	Diptera	<i>Drosophila prosaltans</i>	Present work
wPsd*	A	Diptera	<i>Drosophila pseudosaltans</i>	Present work
wRec	A	Diptera	<i>Drosophila recens</i>	GCF_000742435.1
wSan_Quija	A	Diptera	<i>Drosophila santomea</i>	GCF_005862095.1
wSan	A	Diptera	<i>Drosophila santomea</i>	GCF_018467135.1
wSh	A	Diptera	<i>Drosophila sechellia</i>	GCF_014354315.1
wSpt*	A	Diptera	<i>Drosophila septentriosaltans</i>	Present work
wRi	A	Diptera	<i>Drosophila simulans</i>	GCF_000022285.1
wAu	A	Diptera	<i>Drosophila simulans</i>	GCF_018690095.1
wHa	A	Diptera	<i>Drosophila simulans</i>	GCF_000376605.1
wStv	A	Diptera	<i>Drosophila sturtevantii</i>	GCF_014107475.1
wStv*	A	Diptera	<i>Drosophila sturtevantii1</i>	Present work
wStv*	A	Diptera	<i>Drosophila sturtevantii2</i>	Present work
wStv*	A	Diptera	<i>Drosophila sturtevantii3</i>	Present work
wSpc	A	Diptera	<i>Drosophila subpulchrella</i>	GCF_002300525.1
wSuz	A	Diptera	<i>Drosophila suzukii</i>	GCF_000333795.1
wTei	A	Diptera	<i>Drosophila teissieri</i>	GCF_005862135.1
wTri	A	Diptera	<i>Drosophila triauraria</i>	GCF_014129515.1
wTro	A	Diptera	<i>Drosophila tropicalis</i>	GCF_014129525.1
wYak_KB166	A	Diptera	<i>Drosophila yakuba</i>	GCF_018467115.1
wYak	A	Diptera	<i>Drosophila yakuba</i>	GCF_005862115.1
wGmm	A	Diptera	<i>Glossina morsitans morsitans</i>	GCF_000689175.1
wIrr	A	Diptera	<i>Haematobia irritans</i>	GCF_009732755.1
wCer1	A	Diptera	<i>Rhagoletis cerasi</i>	GCF_018454475.1
wCin2	A	Diptera	<i>Rhagoletis cingulata</i>	GCF_017604245.1
wDacA	A	Hemiptera	<i>Dactylopius coccus</i>	GCF_001648025.1
wAgra	A	Hymenoptera	<i>Anoplolepis gracilipes</i>	GCF_014333535.1
wAdent	A	Hymenoptera	<i>Apterostigma dentigerum</i>	GCF_010820705.1
wCobs-BR2009-alpha	A	Hymenoptera	<i>Cardiocondyla obscurior</i>	GCF_902713635.1
wCobs-JP2010-OypB	A	Hymenoptera	<i>Cardiocondyla obscurior</i>	GCF_902713645.1
wCsol	A	Hymenoptera	<i>Ceratosolen solmsi</i>	GCF_017869155.1
wKgib	A	Hymenoptera	<i>Kradibia gibbosae</i>	GCF_016031645.1
wUni	A	Hymenoptera	<i>Muscidifurax uniraptor</i>	GCF_000174095.1
wUni	A	Hymenoptera	<i>Muscidifurax uniraptor</i>	GCF_001983635.1
wOneA1	A	Hymenoptera	<i>Nasonia oneida</i>	GCF_009012935.1
wVitA	A	Hymenoptera	<i>Nasonia vitripennis</i>	GCF_001983615.1
wNfe	A	Hymenoptera	<i>Nomada ferruginata</i>	GCF_001675785.1
wNfla	A	Hymenoptera	<i>Nomada flava</i>	GCF_001675695.1
wNleu	A	Hymenoptera	<i>Nomada leucophthalma</i>	GCF_001675715.1
wNpa	A	Hymenoptera	<i>Nomada panzeri</i>	GCF_001675775.1
wWpum	A	Hymenoptera	<i>Wiebesia pumilae</i>	GCF_017869285.1
wCauA	A	Lepidoptera	<i>Carposina sasakii</i>	GCF_006542295.1
wMau	B	Diptera	<i>Drosophila mauritiana</i>	GCF_004795955.1
wNo	B	Diptera	<i>Drosophila simulans</i>	GCF_000376585.1
wAlbB	B	Diptera	<i>Aedes albopictus</i>	GCF_004795415.1
wPipPel	B	Diptera	<i>Culex quinquefasciatus</i>	GCF_000073005.1

Supplementary Table S2. Host Information and its GenBank accession number. (Apêndice B)

Supplementary Table S3. Estimates of divergence time among *Wolbachia* strains. (Apêndice C)

Supplementary Table S4. P-distances of *Wolbachia* strains based on third-position substitution rate. (Apêndice D)

Supplementary Table S5. Estimates of divergence time among host species. (Apêndice E)

Supplementary Table S6. P-distances of host species based on substitution rate of all three codon positions of the 13 mitochondrial genes. (Apêndice F)

5. DISCUSSÃO GERAL

A cepa *wStv* foi observada infectando as 14 populações que apresentaram infecção por *Wolbachia*, com prevalência média de 41% e em taxas altamente variáveis (0 – 70%). Ainda, essa cepa foi encontrada sem variabilidade genética, observada a partir da sequência do gene *16S rRNA*. Esse mesmo padrão já foi observado anteriormente pela cepa *wSuz*, em populações de *Drosophila suzukii* (CATTEL et al., 2016). A alta variação na taxa de infecção de *Wolbachia* observada, pode ser explicada devido a vários fatores, tais como: ao *background* genético de cada população hospedeira, como já foi observado anteriormente pelo nosso grupo de pesquisa, diferenciação genética nas populações de *D. sturtevantii* aqui estudadas (TRAVA et al., 2021; ZORZATO et al., 2022); eficiência variável na transferência da cepa da mãe para a prole (transmissão vertical); custo-benefício da infecção para a população hospedeira, como observado também no presente trabalho efeitos variados no valor adaptativo do hospedeiro; e ainda fatores ambientais, tais como a temperatura, umidade, dieta, e outros (MOUTON et al., 2006; YAMADA et al., 2007; WALKER et al., 2011; PONTIER, et al., 2015).

Com base na taxa intermediária de infecção, pode-se concluir que essa cepa não está fixada nas populações de *D. sturtevantii*, que seria esperada em casos de mutualismo obrigatório, na qual o hospedeiro passa a depender da *Wolbachia* que promove funções essenciais, como reprodução e nutrição (SICARD et al., 2014; NIKOH et al. 2014). Estas taxas intermediárias podem estar associadas a eventos estocásticos, ou ainda a não indução de IC ou IC parcial, como também observado no presente trabalho (GIORDANO et al., 1995; CATTEL et al., 2016; MEANY et al., 2019).

As variações encontradas, como no efeito da infecção no valor adaptativo, tais como efeitos benéficos/positivos, neutros e negativos; assim como a variação na indução de IC, de maneira parcial ou ainda a não indução, parecem estar sendo influenciadas pelo *background* genético populacional do hospedeiro. A relação do *background* genético do hospedeiro com a variação do efeito da *Wolbachia* no valor adaptativo já foi observada por Serga et al. (2021) e Strunov et al. (2022). E da mesma forma, também foi verificado que a expressão da IC pode ser afetada pelo *background* genético do hospedeiro, como no caso quando a cepa *wMel* foi transinfectada em *D. simulans* levando a indução de IC, o que não foi observado em *D. melanogaster*, seu hospedeiro natural (POINSOT et al., 1998; GAZLA; CARRECEDO, 2009).

Além dessa variação, foi verificado uma associação da prevalência da *Wolbachia* e efeitos no valor adaptativo do hospedeiro. Os efeitos no valor adaptativo foram benéficos

(7), neutros (18) e negativos (5). Assim, populações com maior prevalência apresentarem mais efeitos benéficos e as com menor prevalência apresentarem mais efeitos negativos. É presumido que com o aumento dos efeitos benéficos ao valor adaptativo do hospedeiro, a infecção por *Wolbachia* consiga se manter e se espalhar nas populações hospedeiras, como já foi observado com a cepa *wAu* e *wRi* em *D. simulans* (HOFFMANN; TURELLI, 1997; KRIESNER et al., 2013).

No presente trabalho, não foi observado efeito da infecção por *Wolbachia* no tempo de desenvolvimento do hospedeiro e nem a indução da manipulação reprodutiva *male-killing* (MK). A explicação para a ausência de efeito no tempo de desenvolvimento pode estar relacionado com a atividade diferencial da *Wolbachia* nos estágios iniciais do desenvolvimento, a qual pode estar ocorrendo uma possível dormência da bactéria nesta etapa (STRUNOV et al., 2022). E também não foi observada associação da cepa *wStv* com os haplótipos do mtDNA das populações hospedeiras, como questionada por Zorzato et al. (2022).

Com base nesses resultados, em relação ao efeito da infecção da cepa *wStv* no hospedeiro natural *D. sturtevantii* e a observação apriori da aquisição horizontal dessa cepa (MILLER; RIEGLER, 2006), fomos motivados a analisar as filogenias e tempos de divergência das cepas do supergrupo A, a qual *wStv* e a maioria das cepas infectantes de *Drosophila* estão inseridas, e de seus hospedeiros, a fim de avaliar a forma de aquisição da *Wolbachia*. Portanto, observamos que, todos os clados compostos de cepas infectantes de *Drosophila*, incluindo *D. sturtevantii*, apresentaram incongruências filogenéticas e de tempo de divergência entre cepas e hospedeiros, demonstrando alta frequência de ocorrências de aquisições horizontais. Os resultados indicaram a ocorrência de pelo menos oito invasões independentes de cepas de *Wolbachia* no gênero *Drosophila* e que após esses eventos, as cepas continuaram se espalhando por outras transmissões horizontais.

Assim, os resultados apresentados aqui demonstram claramente que a transmissão horizontal é um importante meio de dispersão da *Wolbachia* nos hospedeiros desse gênero. Mas, algumas questões ainda permanecem em aberto em relação às rotas dessas transmissões. Há a probabilidade da transmissão horizontal ocorrer do modo direto, devido a sobreposição geográfica de algumas espécies hospedeiras e ao compartilhamento de nichos, como algumas espécies de *Drosophila* e no caso, da não sobreposição de nichos, as transmissões podem ser explicadas pela existência de hospedeiros intermediários e elos perdidos. A ampliação de estudos ecológicos entre os

hospedeiros de *Wolbachia* e o sequenciamento de genomas em larga escala deverão ser pontos cruciais para o esclarecimento das possibilidades das rotas de transmissão.

Por fim, nossos resultados, inéditos para *D. sturtevantii*, espécie neotropical, com ampla distribuição geográfica e uma história evolutiva ainda em construção, corroborou alguns dos resultados já obtidos na literatura para outras espécies do gênero, indicando que o *modo operandi* da *Wolbachia* possa ser semelhante em diferentes espécies relacionadas, o entendimento desse processo é fundamental, principalmente porque esta bactéria tem sido utilizada no controle biológico de pragas na agricultura (BOURTZIS, 2008; NIKOLOULI et al., 2018; MATEOS et al., 2020) e no controle de doenças transmitidas por vetores (WALKER et al., 2011; DUTRA et al., 2016; O'NEILL et al., 2019).

6. CONSIDERAÇÕES FINAIS

Os principais achados do trabalho foram:

- A cepa wStv foi identificada como a única infectante das 14 populações de *D. sturtevantii*, encontrada sem variabilidade genética pelo marcador 16S rRNA;
- A infecção apresentou taxas variáveis (0-70%), com prevalência média de 41%;
- Efeitos positivos/benéficos, neutros e negativos no valor adaptativo foram observados entre as populações hospedeiras de *D. sturtevantii*;
- As populações com maior prevalência apresentaram mais efeitos benéficos e as com menor prevalência mais efeitos negativos em relação aos parâmetros do valor adaptativo avaliados;
- A incompatibilidade citoplasmática foi induzida de modo parcial nas populações de CUR e VIT e nas demais populações não foi observada;
- Houve correlação entre a variação do efeito da infecção no valor adaptativo e manipulação reprodutiva (IC), com o *background* genético das populações hospedeiras;
- Não foi observado efeito direto da *Wolbachia* no parâmetro de tempo de desenvolvimento e na indução da manipulação male-killing nas populações analisadas;
- Os nossos resultados indicam fortemente que as aquisições de wStv, por *D. sturtevantii*, assim como as demais cepas das espécies do gênero *Drosophila* avaliadas ocorreram por transmissão horizontal.

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Apêndice A - Probability p values. Smaller than 0.05 ($p < 0.05$) show statistically significant difference (red). F = female; M = male; IN = infected; UN = uninfected.

Fecundity

FxM	FxM	CANT	CUR	ELD	TIR	VIT
INxIN	UNxUN	0,001	0,011	1,000	0,013	0,000

CI

FxM	FxM	CANT	CUR	ELD	TIR	VIT
UNxIN	INxIN	0,633	0,010	0,000	1,000	0,000
UNxIN	UNxUN	0,039	0,000	0,010	0,004	0,000
UNxIN	INxUN	0,063	0,000	0,000	0,606	0,000

MK

FxM	FxM	CANT	CUR	ELD	TIR	VIT
INxIN-F	INxIN-M	0,007	1,000	0,230	0,000	0,000
UNxUN-F	UNxUN-M	0,565	0,108	1,000	1,000	0,271
INxUN-F	ZINxUN-M	0,181	0,029	1,000	0,001	0,000

Longevity

	FxM	FxM	CANT	CUR	ELD	TIR	VIT
F	INxIN	UNxUN	0,000	0,714	0,000	0,001	0,968
M	INxIN	UNxUN	0,006	0,075	0,000	0,000	0,100

Development time

FxM	FxM	CANT	CUR	ELD	TIR	VIT
INxIN	UNxUN	0,129	0,844	0,939	0,708	0,723

Apêndice B - Host Information and its GenBank accession number.

Host Order	Host family	Host subgenus	Host group	Host species	COI
Diptera	Culicidae	Culex	-	Culex quinquefasciatus	GU188856
Diptera	Culicidae	Stegomyia	-	Aedes albopictus	NC_006817.1
Diptera	Drosophilidae	Drosophila	cardini	Drosophila arawakana	HM006881.1
Diptera	Drosophilidae	Drosophila	flavopilosa	Drosophila incompta	NC_025936
Diptera	Drosophilidae	Drosophila	quinaria	Drosophila recens	AY154456.1
Diptera	Drosophilidae	Drosophila	testacea	Drosophila neotestacea	MN983077.1
Diptera	Drosophilidae	Drosophila	testacea	Drosophila orientacea	AB932743.1
Diptera	Drosophilidae	Drosophila	virilis	Drosophila borealis	HQ849823
Diptera	Drosophilidae	Sophophora	melanogaster	Drosophila ananassae	MK659805
Diptera	Drosophilidae	Sophophora	melanogaster	Drosophila auraria	MK659808
Diptera	Drosophilidae	Sophophora	melanogaster	Drosophila baimaii	AB669750.1
Diptera	Drosophilidae	Sophophora	melanogaster	Drosophila bicornuta	AB669752.1
Diptera	Drosophilidae	Sophophora	melanogaster	Drosophila mauritiana	NC_005779
Diptera	Drosophilidae	Sophophora	melanogaster	Drosophila melanogaster	U37541
Diptera	Drosophilidae	Sophophora	melanogaster	Drosophila nikananu	AB669744.1
Diptera	Drosophilidae	Sophophora	melanogaster	Drosophila santomea	NC_023825
Diptera	Drosophilidae	Sophophora	melanogaster	Drosophila sechellia	MK659840
Diptera	Drosophilidae	Sophophora	melanogaster	Drosophila simulans	AY518674
Diptera	Drosophilidae	Sophophora	melanogaster	Drosophila subpulchrella	KJ463785.1
Diptera	Drosophilidae	Sophophora	melanogaster	Drosophila suzukii	KU588141
Diptera	Drosophilidae	Sophophora	melanogaster	Drosophila teissieri	MK659845
Diptera	Drosophilidae	Sophophora	melanogaster	Drosophila triauraria	MK659846
Diptera	Drosophilidae	Sophophora	melanogaster	Drosophila yakuba	NC_001322
Diptera	Drosophilidae	Sophophora	obscura	Drosophila bifasciata	MK659810
Diptera	Drosophilidae	Sophophora	saltans	Drosophila lehrmanae	New_seq
Diptera	Drosophilidae	Sophophora	saltans	Drosophila prosaltans	New_seq
Diptera	Drosophilidae	Sophophora	saltans	Drosophila pseudosaltans	New_seq
Diptera	Drosophilidae	Sophophora	saltans	Drosophila septentrionsaltans	New_seq
Diptera	Drosophilidae	Sophophora	saltans	Drosophila sturtevantii	New_seq
Diptera	Drosophilidae	Sophophora	willistoni	Drosophila tropicalis	MG010092.1
Diptera	Glossinidae	Glossina	-	Glossina morsitans	EU591836.1
Diptera	Muscidae	-	-	Haematobia irritans	DQ029097
Diptera	Tephritidae	-	-	Rhagoletis cerasi	NC_061399
Diptera	Tephritidae	-	-	Rhagoletis cingulata	DQ006863.1
Diptera	Tephritidae	Ceratitis	-	Ceratitis capitata	NC_000857
Hemiptera	Dactylopiidae	-	-	Dactylopius coccus	MG869728.1
Hymenoptera	Agaonidae	-	-	Ceratosolen solmsi	JF816395.1
Hymenoptera	Agaonidae	-	-	Kradibia gibbosae	MT947598
Hymenoptera	Agaonidae	-	-	Wiebesia pumilae	MT947601
Hymenoptera	Apidae	-	-	Nomada ferruginata	KJ836584.1
Hymenoptera	Apidae	-	-	Nomada flava	KT164670
Hymenoptera	Apidae	-	-	Nomada leucophthalma	MZ626167.1
Hymenoptera	Apidae	-	-	Nomada panzeri	KT074072.1

Hymenoptera	Formicidae	-	-	Anoplolepis gracilipes	NC_039576
Hymenoptera	Formicidae	-	-	Apterostigma dentigerum	BK012289.1
Hymenoptera	Formicidae	-	-	Cardiocondyla obscurior	KX951753
Hymenoptera	Pteromalidae	-	-	Muscidifurax uniraptor	DQ195103.1
Hymenoptera	Pteromalidae	-	-	Nasonia oneida	GU201924.1
Hymenoptera	Pteromalidae	-	-	Nasonia vitripennis	MT985330
Lepidoptera	Carposinidae	-	-	Carposina sasakii	NC_023212

COII	COIII	ND5	ND1	ND2	ND3	ND4
GU188856	GU188856	GU188856	GU188856	GU188856	GU188856	GU188856
NC_006817.1	NC_006817.1	NC_006817.1	NC_006817.1	NC_006817.1	NC_006817.1	NC_006817.1
HM006889.1	-	-	AF246516.1	-	-	-
NC_025936	NC_025936	NC_025936	NC_025936	NC_025936	NC_025936	NC_025936
AF147123.1	AF147133.1	-	-	-	-	-
AF183990.1	AF183994.1	-	-	-	-	-
AF519334.1	AF519366.1	-	-	-	-	-
HQ849823	HQ849823	HQ849823	HQ849823	HQ849823	HQ849823	HQ849823
MK659805	MK659805	MK659805	MK659805	MK659805	MK659805	MK659805
MK659808	MK659808	MK659808	MK659808	MK659808	MK659808	MK659808
EU493754.1	EU493884.1	-	HQ631797.1	-	-	EU494016
AB669809.1	-	-	-	-	-	-
NC_005779	NC_005779	NC_005779	NC_005779	NC_005779	NC_005779	NC_005779
U37541	U37541	U37541	U37541	U37541	U37541	U37541
AF474086.1	-	-	-	-	-	-
NC_023825	NC_023825	NC_023825	NC_023825	NC_023825	NC_023825	NC_023825
MK659840	MK659840	MK659840	MK659840	MK659840	MK659840	MK659840
AY518674	AY518674	AY518674	AY518674	AY518674	AY518674	AY518674
-	-	-	-	-	-	-
KU588141	KU588141	KU588141	KU588141	KU588141	KU588141	KU588141
MK659845	MK659845	MK659845	MK659845	MK659845	MK659845	MK659845
MK659846	MK659846	MK659846	MK659846	MK659846	MK659846	MK659846
NC_001322	NC_001322	NC_001322	NC_001322	NC_001322	NC_001322	NC_001322
MK659810	MK659810	MK659810	MK659810	MK659810	MK659810	MK659810
New_seq	New_seq	New_seq	New_seq	New_seq	New_seq	New_seq
New_seq	New_seq	New_seq	New_seq	New_seq	New_seq	New_seq
-	-	-	-	-	-	-
New_seq	New_seq	New_seq	New_seq	New_seq	New_seq	New_seq
New_seq	New_seq	New_seq	New_seq	New_seq	New_seq	New_seq
AF474103.1	-	-	-	-	-	-
KR820741.1	KC193019.1	KC178125.1	KC176847.1	KC177574.1	KC176824.1	KC177262
DQ029097	DQ029097	DQ029097	DQ029097	DQ029097	DQ029097	DQ029097
NC_061399	NC_061399	NC_061399	NC_061399	NC_061399	NC_061399	NC_061399
AY152492.1	-	-	-	-	-	-
NC_000857	NC_000857	NC_000857	NC_000857	NC_000857	NC_000857	NC_000857
-	-	-	-	-	-	-
JF816395.1	JF816395.1	JF816395.1	JF816395.1	-	-	JF816395.1
MT947598	MT947598	MT947598	MT947598	MT947598	MT947598	MT947598
MT947601	MT947601	MT947601	MT947601	MT947601	MT947601	MT947601
-	-	-	-	-	-	-
KT164670	KT164670	KT164670	KT164670	KT164670	KT164670	KT164670
-	-	-	-	-	-	-
-	-	-	-	-	-	-

ND4L	ND6	ATP8	ATP6	cytB
GU188856	GU188856	GU188856	GU188856	GU188856
NC_006817.1	NC_006817.1	NC_006817.1	NC_006817.1	NC_006817.1
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NC_025936	NC_025936	NC_025936	NC_025936	NC_025936
-	-	-	-	-
-	-	-	-	-
-	-	-	-	-
HQ849823	HQ849823	HQ849823	HQ849823	HQ849823
MK659805	MK659805	MK659805	MK659805	MK659805
MK659808	MK659808	MK659808	MK659808	MK659808
AY958401.1	EU494016	EU493754	EU493754	EU494138.1
-	-	-	-	-
NC_005779	NC_005779	NC_005779	NC_005779	NC_005779
U37541	U37541	U37541	U37541	U37541
-	-	-	-	-
NC_023825	NC_023825	NC_023825	NC_023825	NC_023825
MK659840	MK659840	MK659840	MK659840	MK659840
AY518674	AY518674	AY518674	AY518674	AY518674
-	XM_037872607.1	-	-	XM_037872607.1
KU588141	KU588141	KU588141	KU588141	KU588141
MK659845	MK659845	MK659845	MK659845	MK659845
MK659846	MK659846	MK659846	MK659846	MK659846
NC_001322	NC_001322	NC_001322	NC_001322	NC_001322
MK659810	MK659810	MK659810	MK659810	MK659810
New_seq	New_seq	New_seq	New_seq	New_seq
New_seq	New_seq	New_seq	New_seq	New_seq
-	-	-	-	New_seq
New_seq	New_seq	New_seq	New_seq	New_seq
New_seq	New_seq	New_seq	New_seq	New_seq
-	-	-	-	MG010110.1
KC178382.1	-	KC178448.1	KC193040.1	KC177594.1
DQ029097	DQ029097	DQ029097	DQ029097	DQ029097
NC_061399	NC_061399	NC_061399	NC_061399	NC_061399
-	-	-	-	DQ006894
NC_000857	NC_000857	NC_000857	NC_000857	NC_000857
-	-	-	-	-
AH016005.2	AH016005.2	JF816395.1	JF816395.1	AH016005.2
MT947598	MT947598	MT947598	MT947598	MT947598
MT947601	MT947601	MT947601	MT947601	MT947601
-	-	-	-	-
KT164670	KT164670	KT164670	KT164670	KT164670
-	-	-	-	-
-	-	-	-	-

NC_039576	NC_039576	NC_039576	NC_039576	NC_039576
BK012288.1	BK012169	BK012167	BK012289	BK012288.1
KX951753	KX951753	KX951753	KX951753	KX951753
-	-	-	-	-
-	-	-	-	-
MT985330	MT985330	MT985330	MT985330	MT985330
NC_023212	NC_023212	NC_023212	NC_023212	NC_023212

Apêndice C- Estimates of divergence time among *Wolbachia* strains.

	wDacA	wRi	wTei	wOrie	wYak_KB166	wRi	wPipPel	wUni
wDacA								
wRi	2.251.307							
wTei	2.355.165	1.079.283						
wOrie	2.057.685	1.532.900	1.459.220					
wYak_KB166	2.353.355	1.079.283	3.614	1.459.220				
wRi	2.255.712	3.614	1.083.276	1.537.050	1.083.276			
wPipPel	7.771.673	7.666.426	7.753.511	7.762.689	7.753.511	7.672.738		
wUni	2.333.602	1.311.087	1.170.068	1.490.488	1.170.068	1.313.070	7.730.158	
wSuz	2.253.285	9.035	1.077.556	1.531.206	1.077.556	12.649	7.674.264	1.310.694
wNo	7.956.538	7.832.457	7.801.182	7.945.983	7.801.182	7.838.817	2.144.222	7.831.536
wHa	2.314.303	1.042.427	1.089.968	1.608.582	1.089.968	1.046.404	7.672.158	1.425.896
wMelPop	2.360.664	1.092.807	74.144	1.474.859	74.144	1.096.425	7.763.343	1.184.531
wGmm	2.537.108	1.427.100	1.223.546	1.605.460	1.223.546	1.431.395	8.059.069	1.316.502
wRec	2.367.837	1.099.169	115.685	1.482.727	115.685	1.102.785	7.790.419	1.183.499
wNfla	2.555.729	1.729.837	1.492.127	1.708.975	1.490.311	1.733.467	7.700.394	1.524.130
wNleu	2.553.945	1.729.158	1.492.287	1.708.371	1.490.478	1.732.776	7.676.746	1.528.146
wNpa	2.571.469	1.724.985	1.492.396	1.722.264	1.490.578	1.728.621	7.714.384	1.541.692
wNfe	2.515.337	1.654.589	1.403.306	1.651.919	1.401.497	1.658.205	7.676.172	1.458.378
wInc	2.372.077	1.094.275	133.070	1.508.578	133.070	1.097.922	7.757.701	1.200.519
wVitA	2.295.238	1.287.185	1.106.236	1.445.306	1.106.236	1.290.801	7.829.575	101.007
wUni	2.347.807	1.338.888	1.156.800	1.492.508	1.156.800	1.342.526	7.865.909	25.747
wSpc	2.253.285	9.035	1.077.556	1.531.206	1.077.556	12.649	7.674.264	1.310.694
wAna_India	2.252.091	0	1.079.659	1.533.433	1.079.659	3.614	7.669.105	1.311.087
wAna_Indonesia	2.252.091	0	1.079.659	1.533.433	1.079.659	3.614	7.669.105	1.311.087
wAna_Hawaii	2.261.048	0	1.085.619	1.540.842	1.085.619	3.640	7.702.674	1.316.918
wAlbB	7.837.924	7.706.395	7.771.006	7.832.904	7.771.006	7.712.710	1.804.393	7.737.622
wMau	7.939.032	7.814.971	7.783.698	7.928.494	7.783.698	7.821.324	2.120.322	7.810.374
wSan_Quija	2.355.985	1.079.659	3.615	1.459.728	3.615	1.083.276	7.756.221	1.170.068
wYak_CY17C	2.354.174	1.079.659	3.615	1.459.728	0	1.083.276	7.756.221	1.170.068
wCauA	2.314.936	1.040.905	1.088.458	1.605.406	1.088.458	1.044.519	7.674.647	1.421.814
wAna	2.252.091	0	1.079.659	1.533.433	1.079.659	3.614	7.669.105	1.311.087
wOneA1	2.376.970	1.071.095	1.025.710	1.599.724	1.023.901	1.074.714	7.714.516	1.244.055
wIrr	2.303.011	960.109	838.840	1.488.374	838.840	963.725	7.742.536	1.153.951
wAdent	2.490.232	2.917.749	2.883.518	3.072.841	2.881.701	2.920.586	7.923.005	2.978.187
wNik	2.424.619	2.821.794	2.851.038	2.988.686	2.849.227	2.826.398	7.928.265	2.895.243
wStv	2.479.105	2.883.532	2.838.533	3.045.113	2.836.722	2.886.349	7.845.552	2.921.304
wTri	2.255.263	14.457	1.079.444	1.533.128	1.079.444	18.072	7.676.654	1.308.818
wTro	2.335.426	1.063.092	70.501	1.443.054	70.501	1.066.708	7.761.938	1.153.857
wNeo	2.060.363	1.538.973	1.461.646	59.597	1.461.646	1.542.589	7.758.720	1.484.663
wBai	2.394.670	1.485.052	1.392.698	1.543.356	1.392.698	1.487.378	7.803.976	1.543.955
wBor	2.367.558	1.094.453	110.333	1.474.639	110.333	1.098.453	7.775.484	1.186.645
wBic	2.530.098	1.207.242	1.091.242	1.732.474	1.091.242	1.210.862	7.851.874	1.411.300
wAra	2.331.805	1.055.860	68.693	1.435.821	68.693	1.059.476	7.758.305	1.151.874
wBif	2.391.940	1.275.671	1.092.888	1.484.202	1.092.888	1.279.290	7.865.280	1.151.083
wAgra	2.308.959	2.646.421	2.619.530	2.793.390	2.617.719	2.647.343	7.870.094	2.704.831
wSh	2.315.281	1.042.867	1.090.428	1.609.261	1.090.428	1.046.482	7.675.413	1.427.996
wMel_CS	2.360.664	1.092.807	74.144	1.474.859	74.144	1.096.425	7.763.343	1.184.531
wMel_PC75	2.362.476	1.094.616	75.952	1.476.668	75.952	1.098.235	7.761.525	1.186.515

wKgib	2.325.969	1.044.597	1.099.388	1.618.182	1.099.388	1.048.211	7.697.016	1.427.879
wMelpop	2.360.664	1.092.807	74.144	1.474.859	74.144	1.096.425	7.763.343	1.184.531
wMelOctoless	2.360.664	1.092.807	74.144	1.474.859	74.144	1.096.425	7.763.343	1.184.531
wMelCS_b	2.360.664	1.092.807	74.144	1.474.859	74.144	1.096.425	7.763.343	1.184.531
wMel	2.360.664	1.092.807	74.144	1.474.859	74.144	1.096.425	7.759.708	1.184.531
wCin2	2.318.959	1.043.126	52.420	1.423.056	52.420	1.046.742	7.748.642	1.133.938
wCsol	2.311.201	1.033.625	1.084.788	1.603.518	1.084.788	1.037.239	7.676.080	1.417.770
wwpum	2.355.165	1.173.291	641.689	1.535.165	641.689	1.176.907	7.739.560	1.229.094
wMel_FFD25	2.360.664	1.092.807	74.144	1.474.859	74.144	1.096.425	7.759.708	1.184.531
wAur	2.255.095	14.456	1.079.364	1.533.014	1.079.364	18.070	7.676.080	1.308.711
wCer4	2.304.821	961.917	840.648	1.490.182	840.648	965.533	7.744.353	1.155.934
wCer1	2.369.369	1.175.712	1.000.235	1.482.948	1.000.235	1.179.330	7.753.480	1.303.366
wSan	2.355.985	1.081.467	5.423	1.461.537	1.808	1.085.084	7.758.038	1.172.051
wAu_KB177	2.331.631	1.059.397	66.880	1.439.330	66.880	1.063.013	7.761.357	1.149.798
wCobs-BR2009-alpha	2.345.283	955.746	819.347	1.562.938	819.347	959.380	7.726.129	1.253.869
wCobs-JP2010-OypB	2.303.931	1.031.236	1.077.189	1.594.735	1.077.189	1.034.867	7.675.959	1.421.122
wLeh*	2.475.480	2.879.910	2.833.099	3.045.113	2.831.287	2.882.725	7.847.368	2.913.354
wPro*	2.236.613	1.069.323	138.043	1.417.712	138.043	1.073.004	7.630.971	1.146.050
wPsd*	2.232.951	1.064.313	127.061	1.405.500	127.061	1.067.995	7.652.331	1.131.037
wSpt*	2.255.839	1.079.395	135.849	1.430.556	135.849	1.083.066	7.663.205	1.153.580
wStv_south*	2.477.354	2.885.415	2.840.415	3.047.001	2.838.604	2.888.233	7.847.564	2.923.371
wStv_central*	2.479.166	2.887.226	2.842.227	3.048.812	2.840.415	2.890.045	7.849.380	2.925.358
wStv_north*	2.477.477	2.883.747	2.838.745	3.045.341	2.836.933	2.886.565	7.846.139	2.921.543

wSuz	wNo	wHa	wMelPop	wGmm	wRec	wNfla	wNleu	wNpa	wNfe
7.840.295									
1.040.698	7.861.712								
1.090.699	7.811.037	1.103.498							
1.425.730	8.138.682	1.406.518	1.241.312						
1.097.062	7.832.595	1.106.236	128.395	1.253.757					
1.727.548	7.820.488	1.720.289	1.509.141	1.774.849	1.515.196				
1.726.878	7.792.829	1.719.645	1.509.242	1.773.975	1.515.275	5.435			
1.722.694	7.838.289	1.699.071	1.509.435	1.760.352	1.515.497	27.238	29.035		
1.652.329	7.801.310	1.645.098	1.420.216	1.724.408	1.426.318	108.703	110.125	132.440	
1.092.150	7.807.810	1.114.169	145.830	1.256.637	156.767	1.512.135	1.515.874	1.506.935	1.426.133
1.285.026	7.918.179	1.348.451	1.117.581	1.268.862	1.129.342	1.506.623	1.506.732	1.523.252	1.439.529
1.336.701	7.960.477	1.402.348	1.168.239	1.334.161	1.176.396	1.556.201	1.559.771	1.572.333	1.492.135
3.613	7.840.295	1.040.698	1.090.699	1.425.730	1.097.062	1.727.548	1.726.878	1.722.694	1.652.329
9.035	7.835.189	1.042.790	1.092.807	1.427.618	1.099.169	1.729.837	1.729.158	1.724.985	1.654.589
9.035	7.835.189	1.042.790	1.092.807	1.427.618	1.099.169	1.729.837	1.729.158	1.724.985	1.654.589
9.100	7.868.081	1.048.481	1.098.865	1.436.464	1.103.448	1.740.523	1.739.802	1.735.652	1.664.692
7.714.223	2.119.055	7.748.359	7.779.021	8.083.111	7.797.555	7.774.605	7.747.090	7.792.344	7.746.511
7.822.807	39.831	7.844.226	7.793.537	8.122.258	7.815.105	7.802.946	7.775.353	7.820.716	7.780.209
1.077.556	7.803.904	1.090.347	74.144	1.223.990	115.685	1.492.127	1.492.287	1.492.396	1.403.306
1.077.556	7.803.904	1.090.347	74.144	1.223.990	115.685	1.490.311	1.490.478	1.490.578	1.401.497
1.038.814	7.867.885	65.047	1.101.607	1.397.404	1.104.346	1.716.745	1.716.113	1.695.521	1.645.179
9.035	7.835.189	1.042.790	1.092.807	1.427.618	1.099.169	1.729.837	1.729.158	1.724.985	1.654.589
1.068.994	7.807.595	1.168.622	1.038.838	1.324.720	1.047.054	1.634.449	1.634.107	1.614.913	1.570.379
958.039	7.802.932	1.077.475	853.557	1.221.848	862.042	1.560.794	1.560.713	1.552.072	1.497.044
2.914.333	8.037.145	3.035.385	2.893.097	3.156.434	2.899.870	3.001.076	2.994.076	3.002.167	2.972.066
2.820.194	8.082.458	2.913.524	2.856.932	3.085.445	2.863.709	3.008.677	2.998.056	3.001.977	2.957.515
2.880.125	7.970.318	2.962.785	2.846.234	3.146.505	2.851.213	2.968.915	2.963.859	2.971.252	2.934.160
12.647	7.842.694	1.042.582	1.092.589	1.427.729	1.098.951	1.729.492	1.728.815	1.724.641	1.654.260
1.060.994	7.802.348	1.071.972	83.192	1.210.423	97.582	1.477.199	1.477.413	1.477.448	1.388.459
1.536.744	7.938.472	1.609.261	1.476.778	1.609.947	1.484.646	1.714.546	1.713.922	1.727.844	1.657.464
1.483.354	7.918.657	1.580.684	1.399.236	1.623.006	1.412.593	1.621.899	1.621.601	1.640.537	1.575.964
1.092.725	7.823.155	1.106.951	123.092	1.241.377	135.656	1.503.993	1.504.112	1.504.279	1.415.070
1.205.102	7.957.472	1.297.546	1.104.404	1.482.147	1.110.761	1.791.300	1.790.404	1.782.892	1.712.135
1.053.764	7.807.789	1.068.356	81.383	1.206.646	93.968	1.473.570	1.473.796	1.473.813	1.384.843
1.273.513	7.944.708	1.244.725	1.109.670	476.182	1.117.831	1.583.679	1.585.326	1.567.708	1.539.700
2.644.808	8.020.787	2.709.372	2.621.616	2.841.928	2.632.202	2.743.442	2.735.567	2.736.645	2.697.171
1.040.776	7.865.042	3.614	1.103.580	1.407.138	1.106.318	1.724.047	1.723.390	1.702.833	1.648.836
1.090.699	7.811.037	1.103.498	0	1.241.312	128.395	1.509.141	1.509.242	1.509.435	1.420.216
1.092.508	7.805.592	1.105.307	5.425	1.243.202	130.204	1.510.957	1.511.052	1.511.253	1.422.025

1.042.505	7.888.421	77.701	1.112.543	1.420.212	1.115.274	1.724.133	1.723.475	1.702.918	1.648.918
1.090.699	7.811.037	1.103.498	0	1.241.312	128.395	1.509.141	1.509.242	1.509.435	1.420.216
1.090.699	7.811.037	1.103.498	0	1.241.312	128.395	1.509.141	1.509.242	1.509.435	1.420.216
1.090.699	7.811.037	1.103.498	0	1.241.312	128.395	1.509.141	1.509.242	1.509.435	1.420.216
1.090.699	7.807.407	1.103.498	3.617	1.241.312	128.395	1.509.141	1.509.242	1.509.435	1.420.216
1.041.034	7.796.325	1.052.009	65.102	1.189.558	77.699	1.453.499	1.453.796	1.453.714	1.364.855
1.031.536	7.869.306	63.237	1.097.934	1.403.069	1.100.677	1.711.216	1.710.603	1.689.985	1.636.059
1.171.163	7.854.357	1.126.119	654.635	1.263.198	666.763	1.547.859	1.549.631	1.548.206	1.460.666
1.090.699	7.807.407	1.103.498	3.617	1.241.312	128.395	1.509.141	1.509.242	1.509.435	1.420.216
12.646	7.842.108	1.042.505	1.092.508	1.427.618	1.098.869	1.729.362	1.728.686	1.724.512	1.654.137
959.846	7.804.745	1.079.283	855.365	1.225.625	863.849	1.558.979	1.558.904	1.550.255	1.495.236
1.173.583	7.855.624	1.184.580	1.011.540	1.261.964	1.025.201	1.532.253	1.532.272	1.510.762	1.464.973
1.079.364	7.805.718	1.092.155	75.952	1.225.879	117.492	1.492.127	1.492.287	1.492.396	1.403.306
1.057.300	7.801.765	1.068.277	79.569	1.206.552	93.961	1.473.460	1.473.686	1.473.703	1.384.740
953.667	7.809.856	1.102.758	834.256	1.286.969	840.854	1.599.505	1.599.285	1.588.968	1.538.928
1.029.138	7.859.169	43.567	1.090.395	1.390.283	1.093.156	1.708.378	1.707.773	1.687.043	1.632.878
2.876.502	7.972.133	2.957.352	2.840.796	3.131.369	2.845.779	2.967.098	2.962.048	2.969.432	2.932.350
1.067.185	7.711.651	1.071.001	147.313	1.217.773	158.234	1.429.525	1.428.073	1.429.710	1.337.560
1.062.176	7.727.891	1.065.992	143.700	1.207.109	150.947	1.413.979	1.412.582	1.414.142	1.322.004
1.077.260	7.751.226	1.093.915	148.767	1.238.078	157.823	1.461.975	1.460.411	1.462.205	1.370.166
2.882.008	7.972.332	2.964.670	2.848.118	3.148.479	2.853.096	2.970.806	2.965.743	2.973.146	2.936.043
2.883.819	7.974.147	2.966.481	2.849.931	3.150.371	2.854.907	2.972.623	2.967.554	2.974.965	2.937.853
2.880.340	7.970.914	2.963.006	2.846.447	3.146.751	2.851.426	2.969.137	2.964.080	2.971.475	2.934.379

wInc	wVitA	wUni	wSpc	wAna_India	wAna_Indonesia	wAna_Hawaii	wAlbB	wMau
1.132.007								
1.183.164	79.932							
1.092.150	1.285.026	1.336.701						
1.094.275	1.287.185	1.338.888	9.035					
1.094.275	1.287.185	1.338.888	9.035	0				
1.100.392	1.292.818	1.344.931	9.100	0	0			
7.780.830	7.854.409	7.890.862	7.714.223	7.709.083	7.709.083	7.742.886		
7.790.171	7.900.691	7.942.874	7.822.807	7.817.697	7.817.697	7.850.457	2.093.364	
133.070	1.106.236	1.156.800	1.077.556	1.079.659	1.079.659	1.085.619	7.773.717	7.786.414
133.070	1.106.236	1.156.800	1.077.556	1.079.659	1.079.659	1.085.619	7.773.717	7.786.414
1.112.262	1.344.736	1.398.605	1.038.814	1.040.905	1.040.905	1.046.582	7.745.429	7.850.392
1.094.275	1.287.185	1.338.888	9.035	0	0	0	7.709.083	7.817.697
1.036.225	1.198.958	1.251.945	1.068.994	1.071.095	1.071.095	1.075.175	7.756.234	7.790.108
847.638	1.122.281	1.176.573	958.039	960.109	960.109	965.206	7.760.634	7.787.257
2.894.417	2.946.376	2.997.778	2.914.333	2.916.951	2.916.951	2.932.589	8.024.993	8.019.561
2.867.088	2.902.811	2.953.748	2.820.194	2.822.777	2.822.777	2.834.029	8.024.828	8.061.305
2.849.002	2.908.455	2.955.819	2.880.125	2.882.725	2.882.725	2.898.068	7.952.563	7.952.792
1.094.055	1.286.930	1.338.620	12.647	14.457	14.457	14.562	7.716.613	7.825.205
113.027	1.091.477	1.141.935	1.060.994	1.063.092	1.063.092	1.068.931	7.774.562	7.784.861
1.510.515	1.447.221	1.494.438	1.536.744	1.538.973	1.538.973	1.546.422	7.827.154	7.920.977
1.425.059	1.493.985	1.548.825	1.483.354	1.483.759	1.483.759	1.494.460	7.861.858	7.897.531
153.275	1.121.421	1.172.113	1.092.725	1.094.834	1.094.834	1.100.908	7.786.279	7.805.655
1.102.316	1.367.648	1.419.888	1.205.102	1.207.242	1.207.242	1.212.305	7.911.523	7.939.955
111.204	1.087.863	1.138.299	1.053.764	1.055.860	1.055.860	1.061.647	7.772.749	7.790.302
1.120.397	1.087.082	1.146.661	1.273.513	1.275.671	1.275.671	1.284.871	7.875.930	7.926.161
2.626.231	2.640.597	2.691.718	2.644.808	2.643.721	2.643.721	2.657.330	7.964.955	8.003.265
1.114.253	1.348.552	1.402.453	1.040.776	1.042.867	1.042.867	1.048.559	7.751.641	7.847.548
145.830	1.117.581	1.168.239	1.090.699	1.092.807	1.092.807	1.098.865	7.779.021	7.793.537
147.653	1.119.389	1.170.059	1.092.508	1.094.616	1.094.616	1.100.687	7.773.577	7.788.093

1.123.286	1.353.874	1.407.804	1.042.505	1.044.597	1.044.597	1.050.301	7.773.206	7.870.923
145.830	1.117.581	1.168.239	1.090.699	1.092.807	1.092.807	1.098.865	7.779.021	7.793.537
145.830	1.117.581	1.168.239	1.090.699	1.092.807	1.092.807	1.098.865	7.779.021	7.793.537
145.830	1.117.581	1.168.239	1.090.699	1.092.807	1.092.807	1.098.865	7.779.021	7.793.537
145.830	1.117.581	1.168.239	1.090.699	1.092.807	1.092.807	1.098.865	7.775.391	7.789.908
94.790	1.071.519	1.121.849	1.041.034	1.043.126	1.043.126	1.048.822	7.763.101	7.778.840
1.106.736	1.339.247	1.393.079	1.031.536	1.033.625	1.033.625	1.039.249	7.755.921	7.851.814
678.110	1.170.902	1.225.489	1.171.163	1.173.291	1.173.291	1.178.103	7.792.115	7.838.677
145.830	1.117.581	1.168.239	1.090.699	1.092.807	1.092.807	1.098.865	7.775.391	7.789.908
1.093.973	1.286.834	1.338.520	12.646	14.456	14.456	14.560	7.716.036	7.824.620
849.461	1.124.088	1.178.391	959.846	961.917	961.917	967.028	7.762.448	7.789.070
1.034.245	1.254.831	1.309.978	1.173.583	1.175.712	1.175.712	1.182.366	7.809.673	7.838.119
134.893	1.108.044	1.158.619	1.079.364	1.081.467	1.081.467	1.087.441	7.775.531	7.788.227
109.373	1.087.782	1.138.213	1.057.300	1.059.397	1.059.397	1.065.209	7.773.981	7.784.280
820.822	1.216.787	1.268.285	953.667	955.746	955.746	960.837	7.783.755	7.794.104
1.101.009	1.341.930	1.396.035	1.029.138	1.031.236	1.031.236	1.036.870	7.745.249	7.841.595
2.843.519	2.903.022	2.950.352	2.876.502	2.879.101	2.879.101	2.894.418	7.954.379	7.954.607
170.684	1.078.198	1.127.299	1.067.185	1.069.323	1.069.323	1.075.314	7.664.472	7.695.725
163.372	1.069.512	1.118.670	1.062.176	1.064.313	1.064.313	1.070.270	7.676.808	7.711.956
170.298	1.088.244	1.137.335	1.077.260	1.079.395	1.079.395	1.085.444	7.703.585	7.735.334
2.850.901	2.910.339	2.957.715	2.882.008	2.884.609	2.884.609	2.899.966	7.954.577	7.954.805
2.852.728	2.912.150	2.959.538	2.883.819	2.886.421	2.886.421	2.901.791	7.956.392	7.956.620
2.849.216	2.908.673	2.956.041	2.880.340	2.882.940	2.882.940	2.898.286	7.953.158	7.953.387

wSan_Quija wYak_CY17C wCauA wAna wOneA1 wLrr wAdent wNik wStv wTri

3.615									
1.088.458	1.088.458								
1.079.659	1.079.659	1.040.905							
1.025.710	1.023.901	1.164.917	1.071.095						
838.840	838.840	1.077.395	960.109	960.395					
2.882.708	2.880.891	3.039.852	2.916.951	2.914.552	2.962.091				
2.852.031	2.850.220	2.910.846	2.822.777	2.829.456	2.871.378	1.565.127			
2.837.710	2.835.898	2.967.219	2.882.725	2.853.167	2.904.179	606.063	1.500.567		
1.079.444	1.079.444	1.040.698	14.457	1.070.882	959.918	2.916.150	2.822.215	2.882.151	
70.501	70.501	1.070.084	1.063.092	1.007.345	822.345	2.872.616	2.831.337	2.824.252	1.062.801
1.461.646	1.461.646	1.605.525	1.538.973	1.608.892	1.490.293	3.077.548	2.991.761	3.050.027	1.538.552
1.391.373	1.391.373	1.582.926	1.483.759	1.410.855	1.356.726	3.069.822	2.953.630	3.002.777	1.485.163
110.372	110.372	1.105.444	1.094.834	1.046.286	861.090	2.905.172	2.865.562	2.851.247	1.094.534
1.091.242	1.091.242	1.297.449	1.207.242	1.022.168	944.469	3.113.543	3.018.851	3.049.951	1.206.912
68.693	68.693	1.066.469	1.055.860	1.003.728	818.730	2.865.348	2.827.716	2.813.383	1.055.571
1.092.888	1.092.888	1.241.014	1.275.671	1.165.787	1.058.299	3.027.584	2.936.863	2.987.863	1.275.322
2.616.820	2.615.009	2.711.923	2.643.721	2.623.281	2.665.185	1.390.577	1.318.410	1.379.359	2.646.619
1.090.428	1.090.428	65.052	1.042.867	1.168.709	1.077.556	3.034.628	2.914.757	2.962.227	1.042.582
74.144	74.144	1.101.607	1.092.807	1.038.838	853.557	2.893.097	2.856.932	2.846.234	1.092.589
75.952	75.952	1.103.416	1.094.616	1.040.648	855.365	2.894.915	2.858.744	2.848.047	1.094.398

1.099.388	1.099.388	77.695	1.044.597	1.170.431	1.082.899	3.047.120	2.925.546	2.972.876	1.044.312
74.144	74.144	1.101.607	1.092.807	1.038.838	853.557	2.893.097	2.856.932	2.846.234	1.092.589
74.144	74.144	1.101.607	1.092.807	1.038.838	853.557	2.893.097	2.856.932	2.846.234	1.092.589
74.144	74.144	1.101.607	1.092.807	1.038.838	853.557	2.893.097	2.856.932	2.846.234	1.092.589
74.144	74.144	1.101.607	1.092.807	1.038.838	853.557	2.893.097	2.856.932	2.846.234	1.092.589
52.420	52.420	1.050.123	1.043.126	987.377	802.404	2.856.263	2.818.454	2.807.738	1.042.919
1.084.788	1.084.788	63.232	1.033.625	1.155.816	1.068.303	3.030.616	2.912.511	2.958.015	1.033.420
641.689	641.689	1.115.191	1.173.291	1.059.713	918.066	2.990.718	2.890.862	2.949.031	1.173.058
74.144	74.144	1.101.607	1.092.807	1.038.838	853.557	2.893.097	2.856.932	2.846.234	1.092.589
1.079.364	1.079.364	1.040.621	14.456	1.070.803	959.846	2.916.150	2.822.005	2.881.936	0
840.648	840.648	1.079.203	961.917	962.203	12.651	2.963.908	2.873.189	2.905.990	961.725
1.000.235	1.000.235	1.188.108	1.175.712	1.114.684	878.874	2.996.244	2.866.945	2.948.687	1.175.479
5.423	1.808	1.090.266	1.081.467	1.025.710	840.648	2.882.708	2.852.031	2.837.710	1.081.252
66.880	66.880	1.066.390	1.059.397	1.003.653	818.669	2.868.982	2.827.505	2.820.419	1.059.186
819.347	819.347	1.102.675	955.746	981.478	419.582	2.922.006	2.867.325	2.904.007	955.555
1.077.189	1.077.189	49.009	1.031.236	1.157.636	1.064.261	3.032.204	2.906.261	2.961.075	1.031.030
2.832.274	2.830.462	2.961.785	2.879.101	2.847.732	2.898.744	606.063	1.496.947	47.032	2.878.528
138.043	138.043	1.070.865	1.069.323	1.019.372	853.856	2.606.870	2.617.172	2.543.978	1.069.106
127.061	127.061	1.065.857	1.064.313	1.006.975	846.902	2.608.854	2.606.134	2.544.003	1.064.097
135.849	135.849	1.093.777	1.079.395	1.040.582	853.474	2.656.051	2.655.395	2.587.700	1.079.177
2.839.593	2.837.781	2.969.105	2.884.609	2.855.049	2.906.063	604.264	1.498.794	1.809	2.884.034
2.841.405	2.839.593	2.970.916	2.886.421	2.856.861	2.907.875	606.078	1.500.604	3.618	2.885.846
2.837.922	2.836.110	2.967.441	2.882.940	2.853.380	2.904.396	604.294	1.498.869	0	2.882.366

wTro	wNeo	wBai	wBor	wBic	wAra	wBif	wAgra	wSh	wMel_CS
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1.444.863									
1.372.802	1.540.274								
85.011	1.476.962	1.406.162							
1.072.771	1.737.905	1.409.466	1.109.973						
50.598	1.437.629	1.369.184	88.628	1.069.153					
1.078.038	1.489.632	1.433.843	1.113.428	1.338.158	1.072.611				
2.601.427	2.797.985	2.797.986	2.635.520	2.852.244	2.594.185	2.704.957			
1.071.972	1.609.261	1.579.425	1.107.336	1.297.546	1.068.356	1.244.725	2.706.694		
83.192	1.476.778	1.399.236	123.092	1.104.404	81.383	1.109.670	2.621.616	1.103.580	
85.000	1.478.588	1.401.047	124.903	1.106.214	83.192	1.111.480	2.623.428	1.105.389	5.425

1.080.930	1.618.182	1.595.589	1.116.300	1.306.497	1.077.315	1.250.059	2.717.355	77.701	1.112.543
83.192	1.476.778	1.399.236	123.092	1.104.404	81.383	1.109.670	2.621.616	1.103.580	0
83.192	1.476.778	1.399.236	123.092	1.104.404	81.383	1.109.670	2.621.616	1.103.580	0
83.192	1.476.778	1.399.236	123.092	1.104.404	81.383	1.109.670	2.621.616	1.103.580	0
83.192	1.476.778	1.399.236	123.092	1.104.404	81.383	1.109.670	2.621.616	1.103.580	3.617
34.335	1.424.971	1.352.906	72.350	1.052.872	30.720	1.058.141	2.585.134	1.052.087	65.102
1.066.416	1.603.638	1.581.038	1.101.771	1.291.956	1.062.801	1.235.525	2.708.168	63.242	1.097.934
623.443	1.540.704	1.407.167	660.191	1.132.470	619.829	1.144.963	2.682.891	1.126.203	654.635
83.192	1.476.778	1.399.236	123.092	1.104.404	81.383	1.109.670	2.621.616	1.103.580	3.617
1.062.801	1.538.552	1.485.163	1.094.534	1.206.912	1.055.571	1.275.322	2.646.619	1.042.582	1.092.508
824.152	1.492.102	1.358.535	862.899	946.278	820.537	1.060.108	2.666.996	1.079.364	855.365
985.496	1.484.867	1.317.584	1.020.791	1.011.917	978.263	1.107.529	2.702.338	1.184.668	1.011.540
72.308	1.463.455	1.393.182	112.181	1.093.051	70.501	1.094.698	2.616.820	1.092.237	75.952
3.614	1.441.246	1.369.184	81.393	1.069.153	46.984	1.074.420	2.597.806	1.068.356	79.569
802.776	1.568.508	1.381.580	839.876	983.664	799.144	1.139.861	2.634.643	1.102.840	834.256
1.058.733	1.593.038	1.573.960	1.094.248	1.285.330	1.055.101	1.230.451	2.700.952	43.570	1.090.395
2.818.818	3.050.027	3.000.965	2.845.809	3.044.514	2.807.948	2.982.424	1.379.359	2.956.792	2.840.796
119.604	1.419.661	1.389.321	152.861	1.109.954	114.084	1.084.678	2.367.165	1.071.082	147.313
112.298	1.407.449	1.380.784	143.722	1.103.121	106.775	1.068.617	2.354.226	1.066.073	143.700
117.458	1.432.500	1.391.254	152.464	1.118.085	111.953	1.100.226	2.413.161	1.093.998	148.767
2.826.134	3.051.915	3.004.662	2.853.130	3.051.839	2.815.265	2.989.750	1.381.204	2.964.113	2.848.118
2.827.946	3.053.728	3.006.474	2.854.943	3.053.651	2.817.076	2.991.563	1.383.014	2.965.925	2.849.931
2.824.463	3.050.255	3.003.001	2.851.460	3.050.179	2.813.593	2.988.086	1.379.462	2.962.449	2.846.447

wMel_PC75 wKgib wMelpop wMelOctoless wMelCS_b wMel wCin2 wCsol wwpum

1.114.352								
5.425	1.112.543							
5.425	1.112.543	0						
5.425	1.112.543	0	0					
1.808	1.112.543	3.617	3.617	3.617				
66.910	1.061.047	65.102	65.102	65.102	65.102			
1.099.743	39.749	1.097.934	1.097.934	1.097.934	1.097.934	1.046.456		
656.443	1.135.157	654.635	654.635	654.635	654.635	603.520	1.120.557	
1.808	1.112.543	3.617	3.617	3.617	0	65.102	1.097.934	654.635
1.094.317	1.044.312	1.092.508	1.092.508	1.092.508	1.092.508	1.042.841	1.033.343	1.172.971
857.174	1.084.707	855.365	855.365	855.365	855.365	804.211	1.070.111	919.873
1.013.349	1.193.622	1.011.540	1.011.540	1.011.540	1.011.540	965.533	1.179.007	985.422
77.760	1.101.196	75.952	75.952	75.952	75.952	54.227	1.086.596	643.497
81.377	1.077.315	79.569	79.569	79.569	79.569	30.718	1.062.722	619.783
836.074	1.108.208	834.256	834.256	834.256	834.256	782.739	1.091.721	880.808
1.092.212	61.720	1.090.395	1.090.395	1.090.395	1.090.395	1.038.680	47.192	1.111.314
2.842.608	2.967.441	2.840.796	2.840.796	2.840.796	2.840.796	2.802.304	2.952.581	2.943.597
149.155	1.080.202	147.313	147.313	147.313	147.313	99.356	1.063.505	682.613
145.542	1.075.198	143.700	143.700	143.700	143.700	92.041	1.058.494	677.419
150.604	1.103.092	148.767	148.767	148.767	148.767	97.263	1.086.436	673.500
2.849.931	2.974.762	2.848.118	2.848.118	2.848.118	2.848.118	2.809.620	2.959.900	2.950.916
2.851.744	2.976.573	2.849.931	2.849.931	2.849.931	2.849.931	2.811.431	2.961.711	2.952.727
2.848.260	2.973.098	2.846.447	2.846.447	2.846.447	2.846.447	2.807.948	2.958.236	2.949.251

wMel_FFD25 wAur wCer4 wCer1 wSan wAu_KB177 wCobs-BR2009-alpha wCobs-JP2010-OypB

1.092.508							
855.365	961.654						
1.011.540	1.175.391	880.683					
75.952	1.081.172	842.456	1.002.044				
79.569	1.059.107	820.476	981.806	68.688			
834.256	955.483	421.398	950.437	821.164	799.084		
1.090.395	1.030.953	1.066.077	1.173.669	1.079.006	1.055.022	1.089.595	
2.840.796	2.878.313	2.900.555	2.945.063	2.832.274	2.814.984	2.898.552	2.953.795
147.313	1.069.025	855.696	1.014.313	139.884	115.915	831.056	1.057.519
143.700	1.064.017	848.743	1.007.434	128.903	108.608	829.546	1.052.480
148.767	1.079.095	855.309	1.022.694	137.685	113.779	839.920	1.080.575
2.848.118	2.883.819	2.907.875	2.950.573	2.839.593	2.822.300	2.905.898	2.962.969
2.849.931	2.885.631	2.909.686	2.952.386	2.841.405	2.824.112	2.907.717	2.964.789
2.846.447	2.882.151	2.906.207	2.948.908	2.837.922	2.820.629	2.904.225	2.961.297

wLeh* wPro* wPsd* wSpt* wStv_south* wStv_central* wStv_north*

2.538.459						
2.538.480	70.052					
2.582.198	62.584	77.336				
45.224	2.545.882	2.545.908	2.589.599			
47.033	2.547.721	2.547.749	2.591.433	0		
45.226	2.544.171	2.544.196	2.587.895	0	1.809	0

Apêndice D - P-distances of *Wolbachia* strains based on third-position substitution rate.

	wDacA	wRi	wTei	wOrie	wYak_KB166	wRi	wPipPel
wDacA		0,0008385	0,0008740	0,0007948	0,0008739	0,0008407	0,0015922
wRi	0,0309330		0,0006017	0,0007174	0,0006021	0,0000358	0,0015588
wTei	0,0323600	0,0148294		0,0006899	0,0000353	0,0006026	0,0015687
wOrie	0,0282726	0,0210620	0,0200497		0,0006898	0,0007185	0,0015891
wYak_KB166	0,0323351	0,0148294	0,0000497	0,0200497		0,0006030	0,0015694
wRi	0,0309935	0,0000497	0,0148842	0,0211191	0,0148842		0,0015575
wPipPel	0,1067828	0,1053367	0,1065332	0,1066594	0,1065332	0,1054234	
wUni	0,0320637	0,0180143	0,0160767	0,0204793	0,0160767	0,0180416	0,1062124
wSuz	0,0309601	0,0001241	0,0148056	0,0210388	0,0148056	0,0001738	0,1054444
wNo	0,1093228	0,1076180	0,1071882	0,1091778	0,1071882	0,1077053	0,0294616
wHa	0,0317985	0,0143229	0,0149762	0,0221019	0,0149762	0,0143776	0,1054155
wMelPop	0,0324355	0,0150152	0,0010187	0,0202646	0,0010187	0,0150649	0,1066683
wGmm	0,0348599	0,0196084	0,0168115	0,0220590	0,0168115	0,0196674	0,1107316
wRec	0,0325341	0,0151026	0,0015895	0,0203727	0,0015895	0,0151523	0,1070404
wNfla	0,0351157	0,0237680	0,0205018	0,0234813	0,0204769	0,0238178	0,1058034
wNleu	0,0350912	0,0237586	0,0205040	0,0234730	0,0204792	0,0238083	0,1054785
wNpa	0,0353320	0,0237013	0,0205055	0,0236639	0,0204805	0,0237512	0,1059956
wNfe	0,0345607	0,0227340	0,0192814	0,0226974	0,0192566	0,0227837	0,1054706
wInc	0,0325923	0,0150353	0,0018284	0,0207279	0,0018284	0,0150855	0,1065908
wVitA	0,0315366	0,0176859	0,0151997	0,0198585	0,0151997	0,0177356	0,1075784
wUni	0,0322589	0,0183963	0,0158944	0,0205071	0,0158944	0,0184463	0,1080776
wSpc	0,0309601	0,0001241	0,0148056	0,0210388	0,0148056	0,0001738	0,1054444
wAna_India	0,0309437	0,0000000	0,0148345	0,0210694	0,0148345	0,0000497	0,1053735
wAna_Indonesia	0,0309437	0,0000000	0,0148345	0,0210694	0,0148345	0,0000497	0,1053735
wAna_Hawaii	0,0310668	0,0000000	0,0149164	0,0211712	0,0149164	0,0000500	0,1058347
wAlbB	0,1076931	0,1058859	0,1067736	0,1076241	0,1067736	0,1059726	0,0247924
wMau	0,1090823	0,1073777	0,1069480	0,1089375	0,1069480	0,1074650	0,0291332
wSan_Quija	0,0323712	0,0148345	0,0000497	0,0200567	0,0000497	0,0148842	0,1065705
wYak_CY17C	0,0323464	0,0148345	0,0000497	0,0200567	0,0000000	0,0148842	0,1065705
wCauA	0,0318072	0,0143020	0,0149554	0,0220583	0,0149554	0,0143517	0,1054496
wAna	0,0309437	0,0000000	0,0148345	0,0210694	0,0148345	0,0000497	0,1053735
wOneA1	0,0326596	0,0147168	0,0140933	0,0219802	0,0140684	0,0147666	0,1059975
wIrr	0,0316434	0,0131919	0,0115257	0,0204503	0,0115257	0,0132416	0,1063824
wAdent	0,0342158	0,0400899	0,0396195	0,0422208	0,0395946	0,0401289	0,1088621
wNik	0,0333143	0,0387714	0,0391733	0,0410645	0,0391484	0,0388347	0,1089344
wStv	0,0340629	0,0396197	0,0390014	0,0418399	0,0389766	0,0396584	0,1077979
wTri	0,0309873	0,0001986	0,0148316	0,0210652	0,0148316	0,0002483	0,1054772
wTro	0,0320888	0,0146069	0,0009687	0,0198276	0,0009687	0,0146566	0,1066490
wNeo	0,0283094	0,0211455	0,0200830	0,0008189	0,0200830	0,0211952	0,1066048
wBai	0,0329028	0,0204046	0,0191357	0,0212057	0,0191357	0,0204366	0,1072266
wBor	0,0325302	0,0150378	0,0015160	0,0202615	0,0015160	0,0150927	0,1068352
wBic	0,0347636	0,0165875	0,0149937	0,0238042	0,0149937	0,0166372	0,1078847
wAra	0,0320390	0,0145075	0,0009438	0,0197282	0,0009438	0,0145572	0,1065991
wBif	0,0328653	0,0175277	0,0150163	0,0203929	0,0150163	0,0175774	0,1080689
wAgra	0,0317251	0,0363618	0,0359923	0,0383812	0,0359675	0,0363745	0,1081351
wSh	0,0318120	0,0143290	0,0149825	0,0221113	0,0149825	0,0143787	0,1054602
wMel_CS	0,0324355	0,0150152	0,0010187	0,0202646	0,0010187	0,0150649	0,1066683
wMel_PC75	0,0324604	0,0150400	0,0010436	0,0202894	0,0010436	0,0150897	0,1066434

wKgib	0,0319588	0,0143528	0,0151056	0,0222338	0,0151056	0,0144024	0,1057570
wMelpop	0,0324355	0,0150152	0,0010187	0,0202646	0,0010187	0,0150649	0,1066683
wMelOctoless	0,0324355	0,0150152	0,0010187	0,0202646	0,0010187	0,0150649	0,1066683
wMelCS_b	0,0324355	0,0150152	0,0010187	0,0202646	0,0010187	0,0150649	0,1066683
wMel	0,0324355	0,0150152	0,0010187	0,0202646	0,0010187	0,0150649	0,1066184
wCin2	0,0318625	0,0143326	0,0007202	0,0195528	0,0007202	0,0143822	0,1064663
wCsol	0,0317559	0,0142020	0,0149050	0,0220323	0,0149050	0,0142517	0,1054693
wWpum	0,0323600	0,0161210	0,0088168	0,0210932	0,0088168	0,0161707	0,1063416
wMel_FFD25	0,0324355	0,0150152	0,0010187	0,0202646	0,0010187	0,0150649	0,1066184
wAur	0,0309850	0,0001986	0,0148305	0,0210636	0,0148305	0,0002483	0,1054693
wCer4	0,0316682	0,0132167	0,0115505	0,0204751	0,0115505	0,0132664	0,1064074
wCer1	0,0325551	0,0161543	0,0137432	0,0203757	0,0137432	0,0162040	0,1065328
wSan	0,0323712	0,0148594	0,0000745	0,0200815	0,0000248	0,0149091	0,1065954
wAu_KB177	0,0320366	0,0145561	0,0009189	0,0197764	0,0009189	0,0146058	0,1066410
wCobs-BR2009-alpha	0,0322242	0,0131319	0,0112578	0,0214748	0,0112578	0,0131819	0,1061570
wCobs-JP2010-OypB	0,0316560	0,0141692	0,0148006	0,0219117	0,0148006	0,0142191	0,1054677
wLeh*	0,0340131	0,0395700	0,0389268	0,0418399	0,0389019	0,0396086	0,1078228
wPro*	0,0307311	0,0146925	0,0018967	0,0194794	0,0018967	0,0147431	0,1048495
wPsd*	0,0306808	0,0146237	0,0017458	0,0193116	0,0017458	0,0146743	0,1051430
wSpt*	0,0309952	0,0148309	0,0018666	0,0196558	0,0018666	0,0148813	0,1052924
wStv_south*	0,0340388	0,0396456	0,0390273	0,0418658	0,0390024	0,0396843	0,1078255
wStv_central*	0,0340637	0,0396705	0,0390522	0,0418907	0,0390273	0,0397092	0,1078505
wStv_north*	0,0340405	0,0396227	0,0390044	0,0418430	0,0389795	0,0396614	0,1078059

wUni	wSuz	wNo	wHa	wMelPop	wGmm	wRec	wNfla	wNleu
0,0009348	0,0008381	0,0015858	0,0008648	0,0008764	0,0009556	0,0008798	0,0009061	0,0009037
0,0006979	0,0000552	0,0015361	0,0005963	0,0006039	0,0007096	0,0006088	0,0007635	0,0007611
0,0006572	0,0006000	0,0015371	0,0005997	0,0001569	0,0006454	0,0001965	0,0006985	0,0006975
0,0007285	0,0007185	0,0015758	0,0007122	0,0006977	0,0007492	0,0006848	0,0007694	0,0007656
0,0006568	0,0006005	0,0015380	0,0006003	0,0001558	0,0006453	0,0001959	0,0006978	0,0006969
0,0006991	0,0000647	0,0015361	0,0005963	0,0006043	0,0007121	0,0006102	0,0007650	0,0007627
0,0016168	0,0015549	0,0008253	0,0015672	0,0015692	0,0016237	0,0015728	0,0015972	0,0015940
	0,0006982	0,0016113	0,0007166	0,0006652	0,0007106	0,0006510	0,0007415	0,0007400
0,0180089		0,0015323	0,0005940	0,0006021	0,0007100	0,0006071	0,0007642	0,0007619
0,1076053	0,1077257		0,0015464	0,0015396	0,0016233	0,0015414	0,0015863	0,0015840
0,0195918	0,0142992	0,1080199		0,0006042	0,0006997	0,0006096	0,0007558	0,0007532
0,0162755	0,0149862	0,1073236	0,0151621		0,0006568	0,0002073	0,0007065	0,0007047
0,0180887	0,0195895	0,1118255	0,0193256	0,0170556		0,0006525	0,0007971	0,0007950
0,0162613	0,0150736	0,1076199	0,0151997	0,0017642	0,0172266		0,0006973	0,0006958
0,0209415	0,0237365	0,1074535	0,0236368	0,0207356	0,0243864	0,0208188		0,0000441
0,0209967	0,0237273	0,1070735	0,0236279	0,0207370	0,0243744	0,0208199	0,0000747	
0,0211828	0,0236698	0,1076981	0,0233452	0,0207396	0,0241872	0,0208229	0,0003743	0,0003989
0,0200381	0,0227030	0,1071900	0,0226036	0,0195138	0,0236934	0,0195976	0,0014936	0,0015131
0,0164951	0,0150061	0,1072793	0,0153087	0,0020037	0,0172662	0,0021540	0,0207767	0,0208281
0,0013878	0,0176563	0,1087958	0,0185277	0,0153556	0,0174342	0,0155172	0,0207010	0,0207025
0,0003538	0,0183663	0,1093770	0,0192683	0,0160516	0,0183314	0,0161637	0,0213822	0,0214312
0,0180089	0,0000496	0,1077257	0,0142992	0,0149862	0,0195895	0,0150736	0,0237365	0,0237273
0,0180143	0,0001241	0,1076555	0,0143279	0,0150152	0,0196155	0,0151026	0,0237680	0,0237586
0,0180143	0,0001241	0,1076555	0,0143279	0,0150152	0,0196155	0,0151026	0,0237680	0,0237586
0,0180945	0,0001250	0,1081074	0,0144061	0,0150984	0,0197370	0,0151614	0,0239148	0,0239049
0,1063149	0,1059934	0,0291158	0,1064625	0,1068837	0,1110619	0,1071384	0,1068231	0,1064450
0,1073145	0,1074854	0,0005473	0,1077797	0,1070832	0,1115998	0,1073795	0,1072125	0,1068333
0,0160767	0,0148056	0,1072256	0,0149814	0,0010187	0,0168176	0,0015895	0,0205018	0,0205040
0,0160767	0,0148056	0,1072256	0,0149814	0,0010187	0,0168176	0,0015895	0,0204769	0,0204792
0,0195357	0,0142733	0,1081047	0,0008937	0,0151361	0,0192003	0,0151737	0,0235881	0,0235794
0,0180143	0,0001241	0,1076555	0,0143279	0,0150152	0,0196155	0,0151026	0,0237680	0,0237586
0,0170933	0,0146880	0,1072764	0,0160569	0,0142736	0,0182016	0,0143865	0,0224573	0,0224526
0,0158553	0,0131635	0,1072123	0,0148045	0,0117279	0,0167882	0,0118445	0,0214453	0,0214442
0,0409203	0,0400429	0,1104304	0,0417062	0,0397511	0,0433694	0,0398442	0,0412348	0,0411386
0,0397806	0,0387495	0,1110530	0,0400318	0,0392542	0,0423940	0,0393474	0,0413392	0,0411933
0,0401387	0,0395729	0,1095122	0,0407087	0,0391073	0,0432330	0,0391757	0,0407929	0,0407234
0,0179832	0,0001738	0,1077586	0,0143251	0,0150122	0,0196170	0,0150996	0,0237632	0,0237539
0,0158540	0,0145781	0,1072043	0,0147289	0,0011431	0,0166312	0,0013408	0,0202967	0,0202996
0,0203993	0,0211149	0,1090746	0,0221113	0,0202909	0,0221207	0,0203990	0,0235579	0,0235493
0,0212139	0,0203813	0,1088024	0,0217186	0,0192255	0,0223001	0,0194090	0,0222849	0,0222808
0,0163045	0,0150140	0,1074902	0,0152095	0,0016913	0,0170565	0,0018639	0,0206649	0,0206665
0,0193913	0,0165581	0,1093357	0,0178283	0,0151745	0,0203647	0,0152619	0,0246125	0,0246002
0,0158268	0,0144787	0,1072790	0,0146792	0,0011182	0,0165793	0,0012911	0,0202469	0,0202500
0,0158159	0,0174981	0,1091603	0,0171025	0,0152469	0,0065427	0,0153590	0,0217597	0,0217824
0,0371644	0,0363397	0,1102056	0,0372268	0,0360210	0,0390481	0,0361665	0,0376949	0,0375867
0,0196207	0,0143003	0,1080657	0,0000497	0,0151632	0,0193341	0,0152008	0,0236884	0,0236794
0,0162755	0,0149862	0,1073236	0,0151621	0,0000000	0,0170556	0,0017642	0,0207356	0,0207370
0,0163027	0,0150111	0,1072488	0,0151869	0,0000745	0,0170816	0,0017890	0,0207606	0,0207618

0,0196191	0,0143240	0,1083869	0,0010676	0,0152863	0,0195137	0,0153239	0,0236896	0,0236805
0,0162755	0,0149862	0,1073236	0,0151621	0,0000000	0,0170556	0,0017642	0,0207356	0,0207370
0,0162755	0,0149862	0,1073236	0,0151621	0,0000000	0,0170556	0,0017642	0,0207356	0,0207370
0,0162755	0,0149862	0,1073236	0,0151621	0,0000000	0,0170556	0,0017642	0,0207356	0,0207370
0,0162755	0,0149862	0,1072738	0,0151621	0,0000497	0,0170556	0,0017642	0,0207356	0,0207370
0,0155803	0,0143038	0,1071215	0,0144546	0,0008945	0,0163445	0,0010676	0,0199711	0,0199752
0,0194802	0,0141733	0,1081243	0,0008689	0,0150856	0,0192782	0,0151233	0,0235121	0,0235037
0,0168878	0,0160918	0,1079189	0,0154729	0,0089947	0,0173563	0,0091613	0,0212676	0,0212919
0,0162755	0,0149862	0,1072738	0,0151621	0,0000497	0,0170556	0,0017642	0,0207356	0,0207370
0,0179817	0,0001738	0,1077506	0,0143240	0,0150111	0,0196155	0,0150985	0,0237614	0,0237521
0,0158825	0,0131883	0,1072372	0,0148294	0,0117527	0,0168401	0,0118693	0,0214204	0,0214193
0,0179083	0,0161250	0,1079363	0,0162761	0,0138986	0,0173394	0,0140863	0,0210532	0,0210534
0,0161040	0,0148305	0,1072506	0,0150062	0,0010436	0,0168436	0,0016143	0,0205018	0,0205040
0,0157982	0,0145273	0,1071963	0,0146781	0,0010933	0,0165780	0,0012910	0,0202453	0,0202484
0,0172282	0,0131034	0,1073074	0,0151519	0,0114627	0,0176830	0,0115533	0,0219772	0,0219742
0,0195262	0,0141404	0,1079850	0,0005986	0,0149820	0,0191025	0,0150200	0,0234731	0,0234648
0,0400295	0,0395231	0,1095371	0,0406340	0,0390325	0,0430250	0,0391010	0,0407679	0,0406985
0,0157467	0,0146631	0,1059581	0,0147155	0,0020241	0,0167322	0,0021741	0,0196417	0,0196217
0,0155404	0,0145943	0,1061812	0,0146467	0,0019744	0,0165857	0,0020740	0,0194281	0,0194089
0,0158502	0,0148016	0,1065018	0,0150304	0,0020441	0,0170112	0,0021685	0,0200875	0,0200660
0,0401671	0,0395988	0,1095398	0,0407346	0,0391331	0,0432601	0,0392015	0,0408189	0,0407493
0,0401944	0,0396237	0,1095648	0,0407594	0,0391581	0,0432861	0,0392264	0,0408438	0,0407742
0,0401420	0,0395759	0,1095204	0,0407117	0,0391102	0,0432364	0,0391786	0,0407959	0,0407265

wNpa	wNfe	wInc	wVitA	wUni	wSpc	wAna_India	wAna_Indonesia
0,0009137	0,0008972	0,0008806	0,0008827	0,0008962	0,0008359	0,0008387	0,0008387
0,0007621	0,0007491	0,0006205	0,0006550	0,0006767	0,0000574	0,0000000	0,0000000
0,0007009	0,0006804	0,0002181	0,0005937	0,0006157	0,0006003	0,0006020	0,0006020
0,0007762	0,0007504	0,0007038	0,0006754	0,0006960	0,0007167	0,0007177	0,0007177
0,0007003	0,0006798	0,0002155	0,0005931	0,0006151	0,0006008	0,0006024	0,0006024
0,0007635	0,0007503	0,0006218	0,0006565	0,0006782	0,0000674	0,0000358	0,0000358
0,0016010	0,0015885	0,0015731	0,0015747	0,0015772	0,0015547	0,0015590	0,0015590
0,0007460	0,0007337	0,0006812	0,0001907	0,0001008	0,0006972	0,0006979	0,0006979
0,0007628	0,0007492	0,0006186	0,0006557	0,0006774	0,0000345	0,0000552	0,0000552
0,0015905	0,0015765	0,0015393	0,0015603	0,0015621	0,0015319	0,0015362	0,0015362
0,0007474	0,0007307	0,0006092	0,0006674	0,0006828	0,0005934	0,0005965	0,0005965
0,0007087	0,0006882	0,0002200	0,0006005	0,0006225	0,0006020	0,0006039	0,0006039
0,0007946	0,0007830	0,0006702	0,0006564	0,0006672	0,0007101	0,0007099	0,0007099
0,0007000	0,0006759	0,0002293	0,0005943	0,0006163	0,0006076	0,0006088	0,0006088
0,0000952	0,0001901	0,0007087	0,0006978	0,0007102	0,0007638	0,0007635	0,0007635
0,0000973	0,0001915	0,0007105	0,0006962	0,0007094	0,0007614	0,0007611	0,0007611
	0,0002114	0,0007087	0,0007017	0,0007146	0,0007622	0,0007621	0,0007621
0,0018197		0,0006933	0,0006899	0,0007049	0,0007486	0,0007491	0,0007491
0,0207053	0,0195951		0,0006239	0,0006444	0,0006190	0,0006205	0,0006205
0,0209295	0,0197791	0,0155538		0,0001625	0,0006551	0,0006550	0,0006550
0,0216039	0,0205019	0,0162567	0,0010983		0,0006765	0,0006767	0,0006767
0,0236698	0,0227030	0,0150061	0,0176563	0,0183663		0,0000574	0,0000574
0,0237013	0,0227340	0,0150353	0,0176859	0,0183963	0,0001241		0,0000000
0,0237013	0,0227340	0,0150353	0,0176859	0,0183963	0,0001241	0,0000000	
0,0238479	0,0228729	0,0151194	0,0177633	0,0184794	0,0001250	0,0000000	0,0000000
0,1070668	0,1064371	0,1069086	0,1079196	0,1084204	0,1059934	0,1059228	0,1059228
0,1074566	0,1069001	0,1070369	0,1085555	0,1091351	0,1074854	0,1074152	0,1074152
0,0205055	0,0192814	0,0018284	0,0151997	0,0158944	0,0148056	0,0148345	0,0148345
0,0204805	0,0192566	0,0018284	0,0151997	0,0158944	0,0148056	0,0148345	0,0148345
0,0232965	0,0226048	0,0152825	0,0184767	0,0192168	0,0142733	0,0143020	0,0143020
0,0237013	0,0227340	0,0150353	0,0176859	0,0183963	0,0001241	0,0000000	0,0000000
0,0221889	0,0215770	0,0142377	0,0164737	0,0172017	0,0146880	0,0147168	0,0147168
0,0213255	0,0205694	0,0116465	0,0154201	0,0161661	0,0131635	0,0131919	0,0131919
0,0412498	0,0408362	0,0397693	0,0404832	0,0411895	0,0400429	0,0400789	0,0400789
0,0412472	0,0406363	0,0393938	0,0398846	0,0405845	0,0387495	0,0387850	0,0387850
0,0408250	0,0403154	0,0391453	0,0399622	0,0406130	0,0395729	0,0396086	0,0396086
0,0236966	0,0227295	0,0150323	0,0176824	0,0183926	0,0001738	0,0001986	0,0001986
0,0203001	0,0190774	0,0015530	0,0149969	0,0156902	0,0145781	0,0146069	0,0146069
0,0237406	0,0227736	0,0207545	0,0198848	0,0205336	0,0211149	0,0211455	0,0211455
0,0225410	0,0216537	0,0195803	0,0205273	0,0212809	0,0203813	0,0203869	0,0203869
0,0206688	0,0194431	0,0021060	0,0154083	0,0161048	0,0150140	0,0150430	0,0150430
0,0244969	0,0235247	0,0151458	0,0187915	0,0195093	0,0165581	0,0165875	0,0165875
0,0202502	0,0190277	0,0015279	0,0149472	0,0156402	0,0144787	0,0145075	0,0145075
0,0215403	0,0211555	0,0153943	0,0149365	0,0157551	0,0174981	0,0175277	0,0175277
0,0376015	0,0370591	0,0360844	0,0362818	0,0369842	0,0363397	0,0363247	0,0363247
0,0233969	0,0226550	0,0153098	0,0185291	0,0192697	0,0143003	0,0143290	0,0143290
0,0207396	0,0195138	0,0020037	0,0153556	0,0160516	0,0149862	0,0150152	0,0150152
0,0207646	0,0195386	0,0020288	0,0153804	0,0160766	0,0150111	0,0150400	0,0150400

0,0233981	0,0226561	0,0154340	0,0186022	0,0193432	0,0143240	0,0143528	0,0143528
0,0207396	0,0195138	0,0020037	0,0153556	0,0160516	0,0149862	0,0150152	0,0150152
0,0207396	0,0195138	0,0020037	0,0153556	0,0160516	0,0149862	0,0150152	0,0150152
0,0207396	0,0195138	0,0020037	0,0153556	0,0160516	0,0149862	0,0150152	0,0150152
0,0207396	0,0195138	0,0020037	0,0153556	0,0160516	0,0149862	0,0150152	0,0150152
0,0199740	0,0187531	0,0013024	0,0147227	0,0154142	0,0143038	0,0143326	0,0143326
0,0232204	0,0224794	0,0152066	0,0184013	0,0191409	0,0141733	0,0142020	0,0142020
0,0212723	0,0200695	0,0093172	0,0160882	0,0168382	0,0160918	0,0161210	0,0161210
0,0207396	0,0195138	0,0020037	0,0153556	0,0160516	0,0149862	0,0150152	0,0150152
0,0236948	0,0227278	0,0150312	0,0176811	0,0183913	0,0001738	0,0001986	0,0001986
0,0213005	0,0205445	0,0116716	0,0154450	0,0161911	0,0131883	0,0132167	0,0132167
0,0207579	0,0201287	0,0142105	0,0172414	0,0179991	0,0161250	0,0161543	0,0161543
0,0205055	0,0192814	0,0018534	0,0152245	0,0159194	0,0148305	0,0148594	0,0148594
0,0202487	0,0190263	0,0015028	0,0149461	0,0156391	0,0145273	0,0145561	0,0145561
0,0218324	0,0211449	0,0112781	0,0167187	0,0174262	0,0131034	0,0131319	0,0131319
0,0231800	0,0224357	0,0151279	0,0184381	0,0191815	0,0141404	0,0141692	0,0141692
0,0408000	0,0402905	0,0390700	0,0398875	0,0405378	0,0395231	0,0395589	0,0395589
0,0196442	0,0183781	0,0023452	0,0148144	0,0154891	0,0146631	0,0146925	0,0146925
0,0194303	0,0181643	0,0022447	0,0146951	0,0153705	0,0145943	0,0146237	0,0146237
0,0200907	0,0188261	0,0023399	0,0149525	0,0156270	0,0148016	0,0148309	0,0148309
0,0408510	0,0403412	0,0391714	0,0399881	0,0406390	0,0395988	0,0396345	0,0396345
0,0408760	0,0403661	0,0391965	0,0400129	0,0406640	0,0396237	0,0396594	0,0396594
0,0408281	0,0403184	0,0391482	0,0399652	0,0406160	0,0395759	0,0396116	0,0396116

wAna_Hawaii	wAlbB	wMau	wSan_Quija	wYak_CY17C	wCauA	wAna	wOneA1
0,0008431	0,0016195	0,0015841	0,0008729	0,0008742	0,0008635	0,0008387	0,0008918
0,0000000	0,0015856	0,0015317	0,0006012	0,0006024	0,0005959	0,0000000	0,0005905
0,0006036	0,0015766	0,0015309	0,0000356	0,0000353	0,0006060	0,0006020	0,0005792
0,0007212	0,0016055	0,0015717	0,0006899	0,0006901	0,0007112	0,0007177	0,0007201
0,0006040	0,0015771	0,0015317	0,0000346	0,0000000	0,0006068	0,0006024	0,0005801
0,0000360	0,0015857	0,0015316	0,0006019	0,0006030	0,0005955	0,0000358	0,0005910
0,0015666	0,0007702	0,0008222	0,0015670	0,0015696	0,0015658	0,0015590	0,0015826
0,0007007	0,0016208	0,0016040	0,0006582	0,0006568	0,0007214	0,0006979	0,0006597
0,0000556	0,0015822	0,0015278	0,0005993	0,0006005	0,0005930	0,0000552	0,0005898
0,0015447	0,0008125	0,0001143	0,0015360	0,0015382	0,0015479	0,0015362	0,0015596
0,0005993	0,0015965	0,0015418	0,0005991	0,0006005	0,0001469	0,0005965	0,0006171
0,0006058	0,0015784	0,0015337	0,0001576	0,0001558	0,0006104	0,0006039	0,0005856
0,0007150	0,0016482	0,0016161	0,0006451	0,0006456	0,0006981	0,0007099	0,0006609
0,0006105	0,0015805	0,0015355	0,0001958	0,0001959	0,0006177	0,0006088	0,0005786
0,0007683	0,0016100	0,0015828	0,0006982	0,0006978	0,0007574	0,0007635	0,0007065
0,0007657	0,0016070	0,0015805	0,0006973	0,0006969	0,0007544	0,0007611	0,0007043
0,0007668	0,0016151	0,0015868	0,0007007	0,0007003	0,0007490	0,0007621	0,0007038
0,0007537	0,0016008	0,0015740	0,0006803	0,0006798	0,0007371	0,0007491	0,0006912
0,0006225	0,0015817	0,0015341	0,0002159	0,0002155	0,0006169	0,0006205	0,0005921
0,0006574	0,0015869	0,0015539	0,0005941	0,0005931	0,0006694	0,0006550	0,0006285
0,0006794	0,0015882	0,0015566	0,0006161	0,0006151	0,0006867	0,0006767	0,0006362
0,0000578	0,0015813	0,0015274	0,0005996	0,0006008	0,0005926	0,0000574	0,0005885
0,0000000	0,0015861	0,0015318	0,0006012	0,0006024	0,0005959	0,0000000	0,0005905
0,0000000	0,0015861	0,0015318	0,0006012	0,0006024	0,0005959	0,0000000	0,0005905
	0,0015966	0,0015404	0,0006028	0,0006040	0,0005990	0,0000000	0,0005928
0,1063873		0,0008083	0,0015749	0,0015776	0,0015922	0,0015861	0,0016145
0,1078653	0,0287628		0,0015297	0,0015319	0,0015432	0,0015318	0,0015541
0,0149164	0,1068109	0,1069853		0,0000346	0,0006050	0,0006012	0,0005797
0,0149164	0,1068109	0,1069853	0,0000497		0,0006068	0,0006024	0,0005801
0,0143800	0,1064222	0,1078644	0,0149554	0,0149554		0,0005959	0,0006167
0,0000000	0,1059228	0,1074152	0,0148345	0,0148345	0,0143020		0,0005905
0,0147729	0,1065707	0,1070361	0,0140933	0,0140684	0,0160060	0,0147168	
0,0132619	0,1066311	0,1069969	0,0115257	0,0115257	0,0148034	0,0131919	0,0131958
0,0402938	0,1102634	0,1101888	0,0396084	0,0395834	0,0417676	0,0400789	0,0400459
0,0389396	0,1102611	0,1107623	0,0391869	0,0391620	0,0399950	0,0387850	0,0388767
0,0398195	0,1092682	0,1092714	0,0389901	0,0389652	0,0407696	0,0396086	0,0392025
0,0002001	0,1060263	0,1075183	0,0148316	0,0148316	0,0142992	0,0001986	0,0147139
0,0146871	0,1068225	0,1069640	0,0009687	0,0009687	0,0147030	0,0146069	0,0138409
0,0212478	0,1075451	0,1088342	0,0200830	0,0200830	0,0220599	0,0211455	0,0221062
0,0205339	0,1080219	0,1085121	0,0191175	0,0191175	0,0217494	0,0203869	0,0193851
0,0151265	0,1069835	0,1072497	0,0015165	0,0015165	0,0151888	0,0150430	0,0143760
0,0166571	0,1087043	0,1090950	0,0149937	0,0149937	0,0178270	0,0165875	0,0140446
0,0145870	0,1067976	0,1070387	0,0009438	0,0009438	0,0146533	0,0145075	0,0137912
0,0176541	0,1082153	0,1089055	0,0150163	0,0150163	0,0170515	0,0175277	0,0160179
0,0365117	0,1094385	0,1099649	0,0359551	0,0359302	0,0372618	0,0363247	0,0360439
0,0144072	0,1065075	0,1078253	0,0149825	0,0149825	0,0008938	0,0143290	0,0160581
0,0150984	0,1068837	0,1070832	0,0010187	0,0010187	0,0151361	0,0150152	0,0142736
0,0151234	0,1068089	0,1070084	0,0010436	0,0010436	0,0151609	0,0150400	0,0142985

0,0144311	0,1068039	0,1081465	0,0151056	0,0151056	0,0010675	0,0143528	0,0160817
0,0150984	0,1068837	0,1070832	0,0010187	0,0010187	0,0151361	0,0150152	0,0142736
0,0150984	0,1068837	0,1070832	0,0010187	0,0010187	0,0151361	0,0150152	0,0142736
0,0150984	0,1068837	0,1070832	0,0010187	0,0010187	0,0151361	0,0150152	0,0142736
0,0150984	0,1068339	0,1070333	0,0010187	0,0010187	0,0151361	0,0150152	0,0142736
0,0144108	0,1066650	0,1068813	0,0007202	0,0007202	0,0144287	0,0143326	0,0135666
0,0142793	0,1065664	0,1078839	0,0149050	0,0149050	0,0008688	0,0142020	0,0158809
0,0161871	0,1070637	0,1077034	0,0088168	0,0088168	0,0153227	0,0161210	0,0145605
0,0150984	0,1068339	0,1070333	0,0010187	0,0010187	0,0151361	0,0150152	0,0142736
0,0002001	0,1060183	0,1075103	0,0148305	0,0148305	0,0142981	0,0001986	0,0147128
0,0132870	0,1066560	0,1070218	0,0115505	0,0115505	0,0148282	0,0132167	0,0132207
0,0162457	0,1073049	0,1076958	0,0137432	0,0137432	0,0163246	0,0161543	0,0153158
0,0149414	0,1068358	0,1070102	0,0000745	0,0000248	0,0149802	0,0148594	0,0140933
0,0146360	0,1068145	0,1069560	0,0009189	0,0009189	0,0146522	0,0145561	0,0137902
0,0132019	0,1069488	0,1070910	0,0112578	0,0112578	0,0151508	0,0131319	0,0134855
0,0142466	0,1064197	0,1077435	0,0148006	0,0148006	0,0006734	0,0141692	0,0159059
0,0397693	0,1092932	0,1092963	0,0389154	0,0388905	0,0406949	0,0395589	0,0391278
0,0147748	0,1053098	0,1057393	0,0018967	0,0018967	0,0147137	0,0146925	0,0140062
0,0147055	0,1054793	0,1059623	0,0017458	0,0017458	0,0146449	0,0146237	0,0138358
0,0149140	0,1058473	0,1062835	0,0018666	0,0018666	0,0150285	0,0148309	0,0142976
0,0398455	0,1092959	0,1092990	0,0390160	0,0389911	0,0407955	0,0396345	0,0392284
0,0398706	0,1093208	0,1093240	0,0390409	0,0390160	0,0408204	0,0396594	0,0392533
0,0398225	0,1092764	0,1092795	0,0389931	0,0389682	0,0407726	0,0396116	0,0392054

wIrr	wAdent	wNik	wStv	wTri	wTro	wNeo	wBai	wBor
0,0008769	0,0008854	0,0008745	0,0008695	0,0008339	0,0008789	0,0007999	0,0008721	0,0008847
0,0005652	0,0009853	0,0009679	0,0009821	0,0000709	0,0005973	0,0007207	0,0007021	0,0006034
0,0005248	0,0009615	0,0009752	0,0009648	0,0005999	0,0001522	0,0006892	0,0006511	0,0001955
0,0007099	0,0010002	0,0009992	0,0009956	0,0007150	0,0006876	0,0001421	0,0007332	0,0006940
0,0005245	0,0009612	0,0009741	0,0009644	0,0006004	0,0001498	0,0006888	0,0006504	0,0001952
0,0005680	0,0009866	0,0009691	0,0009830	0,0000799	0,0005981	0,0007216	0,0007019	0,0006046
0,0015566	0,0015838	0,0016092	0,0015895	0,0015582	0,0015708	0,0015894	0,0015777	0,0015816
0,0006545	0,0010086	0,0010267	0,0010032	0,0006971	0,0006459	0,0007273	0,0007257	0,0006475
0,0005649	0,0009819	0,0009675	0,0009797	0,0000669	0,0005955	0,0007219	0,0007003	0,0006016
0,0015344	0,0015626	0,0015995	0,0015773	0,0015340	0,0015422	0,0015763	0,0015753	0,0015477
0,0005923	0,0010126	0,0009649	0,0010001	0,0005963	0,0005958	0,0007136	0,0007083	0,0006070
0,0005335	0,0009659	0,0009780	0,0009623	0,0006019	0,0001663	0,0006967	0,0006526	0,0002030
0,0006614	0,0010378	0,0010313	0,0010122	0,0007081	0,0006506	0,0007485	0,0007433	0,0006559
0,0005320	0,0009629	0,0009758	0,0009597	0,0006067	0,0001789	0,0006842	0,0006442	0,0002155
0,0007238	0,0009837	0,0010053	0,0009732	0,0007607	0,0006973	0,0007680	0,0007245	0,0007068
0,0007229	0,0009803	0,0010017	0,0009718	0,0007583	0,0006961	0,0007641	0,0007236	0,0007051
0,0007264	0,0009842	0,0010055	0,0009775	0,0007597	0,0006992	0,0007759	0,0007358	0,0007084
0,0007037	0,0009703	0,0009957	0,0009649	0,0007462	0,0006796	0,0007494	0,0007119	0,0006896
0,0005461	0,0009743	0,0009883	0,0009785	0,0006193	0,0001985	0,0007033	0,0006684	0,0002309
0,0006104	0,0009478	0,0009667	0,0009501	0,0006557	0,0005891	0,0006779	0,0006836	0,0005899
0,0006277	0,0009632	0,0009777	0,0009615	0,0006778	0,0006106	0,0006980	0,0006923	0,0006119
0,0005656	0,0009808	0,0009646	0,0009781	0,0000662	0,0005955	0,0007201	0,0006998	0,0006014
0,0005652	0,0009851	0,0009682	0,0009819	0,0000709	0,0005973	0,0007207	0,0007013	0,0006036
0,0005652	0,0009851	0,0009682	0,0009819	0,0000709	0,0005973	0,0007207	0,0007013	0,0006036
0,0005690	0,0009907	0,0009749	0,0009876	0,0000714	0,0005990	0,0007242	0,0007066	0,0006056
0,0015791	0,0016299	0,0016180	0,0016212	0,0015836	0,0015795	0,0016058	0,0016081	0,0015855
0,0015292	0,0015604	0,0015998	0,0015721	0,0015295	0,0015363	0,0015721	0,0015735	0,0015418
0,0005234	0,0009602	0,0009740	0,0009632	0,0005991	0,0001495	0,0006888	0,0006507	0,0001944
0,0005245	0,0009608	0,0009745	0,0009641	0,0006004	0,0001498	0,0006888	0,0006509	0,0001953
0,0005894	0,0010191	0,0009604	0,0010052	0,0005960	0,0006021	0,0007124	0,0007111	0,0006137
0,0005652	0,0009851	0,0009682	0,0009819	0,0000709	0,0005973	0,0007207	0,0007013	0,0006036
0,0005761	0,0009826	0,0009623	0,0009688	0,0005897	0,0005720	0,0007238	0,0006432	0,0005826
	0,0009764	0,0009778	0,0009713	0,0005650	0,0005251	0,0007074	0,0006404	0,0005368
0,0406991		0,0007286	0,0004629	0,0009791	0,0009639	0,0010029	0,0009905	0,0009699
0,0394527	0,0215048		0,0007250	0,0009665	0,0009754	0,0010030	0,0009729	0,0009808
0,0399034	0,0083273	0,0206178		0,0009773	0,0009603	0,0009997	0,0009848	0,0009683
0,0131893	0,0400679	0,0387772	0,0396008		0,0005953	0,0007182	0,0006995	0,0006019
0,0112990	0,0394697	0,0389026	0,0388052	0,0146029		0,0006864	0,0006405	0,0001796
0,0204766	0,0422855	0,0411068	0,0419074	0,0211397	0,0198524		0,0007321	0,0006917
0,0186414	0,0421793	0,0405829	0,0412581	0,0204061	0,0188623	0,0211634		0,0006495
0,0118314	0,0399171	0,0393728	0,0391761	0,0150389	0,0011681	0,0202935	0,0193207	
0,0129770	0,0427801	0,0414790	0,0419063	0,0165830	0,0147399	0,0238788	0,0193661	0,0152510
0,0112493	0,0393699	0,0388528	0,0386559	0,0145036	0,0006952	0,0197530	0,0188126	0,0012178
0,0145410	0,0415990	0,0403525	0,0410532	0,0175229	0,0148122	0,0204675	0,0197010	0,0152985
0,0366196	0,0191065	0,0181150	0,0189524	0,0363645	0,0357436	0,0384443	0,0384443	0,0362120
0,0148056	0,0416958	0,0400488	0,0407010	0,0143251	0,0147289	0,0221113	0,0217013	0,0152148
0,0117279	0,0397511	0,0392542	0,0391073	0,0150122	0,0011431	0,0202909	0,0192255	0,0016913
0,0117527	0,0397761	0,0392791	0,0391322	0,0150370	0,0011679	0,0203158	0,0192504	0,0017162

0,0148790	0,0418674	0,0401970	0,0408473	0,0143488	0,0148520	0,0222338	0,0219234	0,0153380
0,0117279	0,0397511	0,0392542	0,0391073	0,0150122	0,0011431	0,0202909	0,0192255	0,0016913
0,0117279	0,0397511	0,0392542	0,0391073	0,0150122	0,0011431	0,0202909	0,0192255	0,0016913
0,0117279	0,0397511	0,0392542	0,0391073	0,0150122	0,0011431	0,0202909	0,0192255	0,0016913
0,0117279	0,0397511	0,0392542	0,0391073	0,0150122	0,0011431	0,0202909	0,0192255	0,0016913
0,0110250	0,0392451	0,0387256	0,0385783	0,0143297	0,0004718	0,0195791	0,0185889	0,0009941
0,0146785	0,0416407	0,0400179	0,0406431	0,0141992	0,0146526	0,0220340	0,0217235	0,0151383
0,0126142	0,0410925	0,0397204	0,0405197	0,0161178	0,0085661	0,0211693	0,0193345	0,0090710
0,0117279	0,0397511	0,0392542	0,0391073	0,0150122	0,0011431	0,0202909	0,0192255	0,0016913
0,0131883	0,0400679	0,0387743	0,0395978	0,0000000	0,0146029	0,0211397	0,0204061	0,0150389
0,0001738	0,0407241	0,0394776	0,0399283	0,0132141	0,0113238	0,0205015	0,0186663	0,0118562
0,0120757	0,0411684	0,0393918	0,0405150	0,0161511	0,0135407	0,0204021	0,0181036	0,0140257
0,0115505	0,0396084	0,0391869	0,0389901	0,0148564	0,0009935	0,0201079	0,0191423	0,0015414
0,0112485	0,0394198	0,0388499	0,0387526	0,0145532	0,0000497	0,0198027	0,0188126	0,0011183
0,0057651	0,0401484	0,0393970	0,0399011	0,0131293	0,0110301	0,0215513	0,0189829	0,0115399
0,0146229	0,0416625	0,0399320	0,0406852	0,0141664	0,0145470	0,0218883	0,0216262	0,0150350
0,0398287	0,0083273	0,0205680	0,0006462	0,0395510	0,0387306	0,0419074	0,0412333	0,0391014
0,0117320	0,0358184	0,0359599	0,0349543	0,0146895	0,0016434	0,0195061	0,0190893	0,0021003
0,0116364	0,0358457	0,0358083	0,0349546	0,0146207	0,0015430	0,0193383	0,0189720	0,0019747
0,0117267	0,0364941	0,0364851	0,0355550	0,0148279	0,0016139	0,0196826	0,0191158	0,0020948
0,0399293	0,0083026	0,0205934	0,0000249	0,0396266	0,0388311	0,0419333	0,0412841	0,0392020
0,0399542	0,0083275	0,0206183	0,0000497	0,0396515	0,0388560	0,0419582	0,0413089	0,0392269
0,0399064	0,0083030	0,0205945	0,0000000	0,0396037	0,0388081	0,0419105	0,0412612	0,0391791

wBic	wAra	wBif	wAgra	wSh	wMel_CS	wMel_PC75	wKgib	wMelpop
0,0009105	0,0008772	0,0009038	0,0008418	0,0008632	0,0008764	0,0008764	0,0008614	0,0008764
0,0006539	0,0005978	0,0006725	0,0009409	0,0005953	0,0006039	0,0006032	0,0005983	0,0006039
0,0006146	0,0001509	0,0005947	0,0009203	0,0005996	0,0001569	0,0001595	0,0006031	0,0001569
0,0007712	0,0006911	0,0007124	0,0009515	0,0007101	0,0006977	0,0006989	0,0007069	0,0006977
0,0006152	0,0001496	0,0005941	0,0009197	0,0006003	0,0001558	0,0001579	0,0006038	0,0001558
0,0006541	0,0005984	0,0006749	0,0009415	0,0005950	0,0006043	0,0006036	0,0005979	0,0006043
0,0015618	0,0015718	0,0015946	0,0016367	0,0015665	0,0015692	0,0015689	0,0015620	0,0015692
0,0007192	0,0006506	0,0006397	0,0009534	0,0007160	0,0006652	0,0006635	0,0007217	0,0006652
0,0006551	0,0005970	0,0006711	0,0009386	0,0005927	0,0006021	0,0006013	0,0005955	0,0006021
0,0015546	0,0015422	0,0015964	0,0016028	0,0015464	0,0015396	0,0015407	0,0015389	0,0015396
0,0006680	0,0005982	0,0006462	0,0009442	0,0000356	0,0006042	0,0006028	0,0001564	0,0006042
0,0006191	0,0001712	0,0005988	0,0009228	0,0006038	0,0000000	0,0000438	0,0006081	0,0000000
0,0007036	0,0006487	0,0004139	0,0010013	0,0006995	0,0006568	0,0006560	0,0006993	0,0006568
0,0006206	0,0001768	0,0005936	0,0009172	0,0006093	0,0002073	0,0002077	0,0006126	0,0002073
0,0007561	0,0006990	0,0007390	0,0009392	0,0007547	0,0007065	0,0007076	0,0007495	0,0007065
0,0007553	0,0006977	0,0007356	0,0009367	0,0007521	0,0007047	0,0007058	0,0007466	0,0007047
0,0007553	0,0007006	0,0007346	0,0009424	0,0007463	0,0007087	0,0007097	0,0007414	0,0007087
0,0007406	0,0006785	0,0007251	0,0009342	0,0007298	0,0006882	0,0006900	0,0007258	0,0006882
0,0006210	0,0001954	0,0006140	0,0009360	0,0006096	0,0002200	0,0002210	0,0006147	0,0002200
0,0006794	0,0005907	0,0005879	0,0009100	0,0006658	0,0006005	0,0006000	0,0006726	0,0006005
0,0006878	0,0006121	0,0005999	0,0009181	0,0006811	0,0006225	0,0006216	0,0006874	0,0006225
0,0006551	0,0005970	0,0006720	0,0009361	0,0005922	0,0006020	0,0006013	0,0005952	0,0006020
0,0006539	0,0005978	0,0006725	0,0009404	0,0005953	0,0006039	0,0006032	0,0005983	0,0006039
0,0006539	0,0005978	0,0006725	0,0009404	0,0005953	0,0006039	0,0006032	0,0005983	0,0006039
0,0006555	0,0005994	0,0006779	0,0009470	0,0005981	0,0006058	0,0006052	0,0006014	0,0006058
0,0015868	0,0015810	0,0016183	0,0016450	0,0015967	0,0015784	0,0015790	0,0015908	0,0015784
0,0015519	0,0015361	0,0015925	0,0015985	0,0015418	0,0015337	0,0015346	0,0015340	0,0015337
0,0006142	0,0001498	0,0005940	0,0009184	0,0005989	0,0001576	0,0001598	0,0006023	0,0001576
0,0006152	0,0001496	0,0005941	0,0009197	0,0006003	0,0001558	0,0001579	0,0006038	0,0001558
0,0006696	0,0006045	0,0006482	0,0009486	0,0001479	0,0006104	0,0006094	0,0001642	0,0006104
0,0006539	0,0005978	0,0006725	0,0009404	0,0005953	0,0006039	0,0006032	0,0005983	0,0006039
0,0005946	0,0005733	0,0005930	0,0009240	0,0006164	0,0005856	0,0005850	0,0006182	0,0005856
0,0005888	0,0005215	0,0005921	0,0009329	0,0005916	0,0005335	0,0005330	0,0005864	0,0005335
0,0010070	0,0009596	0,0009976	0,0006873	0,0010091	0,0009659	0,0009680	0,0010141	0,0009659
0,0010181	0,0009709	0,0009893	0,0006648	0,0009617	0,0009780	0,0009795	0,0009622	0,0009780
0,0010035	0,0009554	0,0009934	0,0006825	0,0009968	0,0009623	0,0009635	0,0010012	0,0009623
0,0006544	0,0005964	0,0006702	0,0009386	0,0005950	0,0006019	0,0006013	0,0005990	0,0006019
0,0006045	0,0001280	0,0005944	0,0009245	0,0005960	0,0001663	0,0001677	0,0005996	0,0001663
0,0007749	0,0006904	0,0007160	0,0009601	0,0007113	0,0006967	0,0006978	0,0007098	0,0006967
0,0006433	0,0006432	0,0006910	0,0009295	0,0007073	0,0006526	0,0006551	0,0007117	0,0006526
0,0006140	0,0001764	0,0005985	0,0009256	0,0006074	0,0002030	0,0002022	0,0006104	0,0002030
	0,0006041	0,0006654	0,0009738	0,0006673	0,0006191	0,0006203	0,0006684	0,0006191
0,0146902		0,0005919	0,0009161	0,0005982	0,0001712	0,0001718	0,0006018	0,0001712
0,0183863	0,0147377		0,0009431	0,0006452	0,0005988	0,0005989	0,0006457	0,0005988
0,0391898	0,0356441	0,0371661		0,0009415	0,0009228	0,0009246	0,0009417	0,0009228
0,0178283	0,0146792	0,0171025	0,0371900		0,0006038	0,0006024	0,0001583	0,0006038
0,0151745	0,0011182	0,0152469	0,0360210	0,0151632		0,0000438	0,0006081	0,0000000
0,0151994	0,0011431	0,0152717	0,0360459	0,0151880	0,0000745		0,0006069	0,0000438

0,0179513	0,0148023	0,0171758	0,0373365	0,0010676	0,0152863	0,0153112	0,0006081
0,0151745	0,0011182	0,0152469	0,0360210	0,0151632	0,0000000	0,0000745	0,0152863
0,0151745	0,0011182	0,0152469	0,0360210	0,0151632	0,0000000	0,0000745	0,0152863
0,0151745	0,0011182	0,0152469	0,0360210	0,0151632	0,0000000	0,0000745	0,0152863
0,0151745	0,0011182	0,0152469	0,0360210	0,0151632	0,0000497	0,0000248	0,0152863
0,0144665	0,0004221	0,0145389	0,0355197	0,0144557	0,0008945	0,0009193	0,0145788
0,0177515	0,0146029	0,0169761	0,0372102	0,0008689	0,0150856	0,0151105	0,0005461
0,0155601	0,0085164	0,0157318	0,0368629	0,0154740	0,0089947	0,0090195	0,0155971
0,0151745	0,0011182	0,0152469	0,0360210	0,0151632	0,0000497	0,0000248	0,0152863
0,0165830	0,0145036	0,0175229	0,0363645	0,0143251	0,0150111	0,0150359	0,0143488
0,0130019	0,0112742	0,0145659	0,0366445	0,0148305	0,0117527	0,0117776	0,0149039
0,0139037	0,0134413	0,0152174	0,0371301	0,0162773	0,0138986	0,0139234	0,0164004
0,0150185	0,0009687	0,0150411	0,0359551	0,0150073	0,0010436	0,0010684	0,0151304
0,0146902	0,0006456	0,0147625	0,0356939	0,0146792	0,0010933	0,0011181	0,0148023
0,0135155	0,0109802	0,0156617	0,0362000	0,0151530	0,0114627	0,0114877	0,0152268
0,0176604	0,0144971	0,0169064	0,0371111	0,0005987	0,0149820	0,0150070	0,0008480
0,0418316	0,0385812	0,0409785	0,0189524	0,0406263	0,0390325	0,0390574	0,0407726
0,0152508	0,0015675	0,0149035	0,0325248	0,0147167	0,0020241	0,0020494	0,0148420
0,0151569	0,0014671	0,0146828	0,0323471	0,0146478	0,0019744	0,0019997	0,0147732
0,0153625	0,0015382	0,0151171	0,0331568	0,0150315	0,0020441	0,0020693	0,0151565
0,0419323	0,0386817	0,0410792	0,0189777	0,0407269	0,0391331	0,0391581	0,0408732
0,0419572	0,0387066	0,0411041	0,0190026	0,0407518	0,0391581	0,0391830	0,0408981
0,0419095	0,0386588	0,0410563	0,0189538	0,0407040	0,0391102	0,0391351	0,0408504

wMelOctoless	wMelCS_b	wMel	wCin2	wCsol	wWpum	wMel_FFD25	wAur	wCer4
0,0008764	0,0008764	0,0008756	0,0008735	0,0008650	0,0008637	0,0008756	0,0008338	0,0008780
0,0006039	0,0006039	0,0006031	0,0005925	0,0005949	0,0006354	0,0006031	0,0000709	0,0005664
0,0001569	0,0001569	0,0001573	0,0001327	0,0006012	0,0004553	0,0001573	0,0005998	0,0005286
0,0006977	0,0006977	0,0006984	0,0006847	0,0007100	0,0007213	0,0006984	0,0007149	0,0007118
0,0001558	0,0001558	0,0001558	0,0001312	0,0006020	0,0004546	0,0001558	0,0006003	0,0005280
0,0006043	0,0006043	0,0006036	0,0005934	0,0005944	0,0006359	0,0006036	0,0000799	0,0005693
0,0015692	0,0015692	0,0015692	0,0015688	0,0015590	0,0015770	0,0015692	0,0015579	0,0015552
0,0006652	0,0006652	0,0006630	0,0006445	0,0007183	0,0006766	0,0006630	0,0006971	0,0006517
0,0006021	0,0006021	0,0006012	0,0005911	0,0005917	0,0006345	0,0006012	0,0000669	0,0005662
0,0015396	0,0015396	0,0015398	0,0015397	0,0015372	0,0015421	0,0015398	0,0015338	0,0015340
0,0006042	0,0006042	0,0006023	0,0005924	0,0001418	0,0006046	0,0006023	0,0005962	0,0005977
0,0000000	0,0000000	0,0000355	0,0001469	0,0006050	0,0004633	0,0000355	0,0006019	0,0005374
0,0006568	0,0006568	0,0006558	0,0006445	0,0006980	0,0006564	0,0006558	0,0007080	0,0006644
0,0002073	0,0002073	0,0002061	0,0001659	0,0006116	0,0004615	0,0002061	0,0006066	0,0005356
0,0007065	0,0007065	0,0007062	0,0006931	0,0007509	0,0007192	0,0007062	0,0007606	0,0007223
0,0007047	0,0007047	0,0007045	0,0006922	0,0007482	0,0007185	0,0007045	0,0007582	0,0007214
0,0007087	0,0007087	0,0007083	0,0006953	0,0007428	0,0007237	0,0007083	0,0007596	0,0007249
0,0006882	0,0006882	0,0006885	0,0006737	0,0007267	0,0006921	0,0006885	0,0007461	0,0007015
0,0002200	0,0002200	0,0002203	0,0001816	0,0006118	0,0004656	0,0002203	0,0006192	0,0005492
0,0006005	0,0006005	0,0005992	0,0005851	0,0006704	0,0006319	0,0005992	0,0006557	0,0006096
0,0006225	0,0006225	0,0006209	0,0006069	0,0006845	0,0006444	0,0006209	0,0006778	0,0006264
0,0006020	0,0006020	0,0006012	0,0005911	0,0005918	0,0006342	0,0006012	0,0000662	0,0005670
0,0006039	0,0006039	0,0006031	0,0005925	0,0005949	0,0006354	0,0006031	0,0000709	0,0005664
0,0006039	0,0006039	0,0006031	0,0005925	0,0005949	0,0006354	0,0006031	0,0000709	0,0005664
0,0006058	0,0006058	0,0006051	0,0005941	0,0005977	0,0006381	0,0006051	0,0000714	0,0005702
0,0015784	0,0015784	0,0015781	0,0015762	0,0015896	0,0015904	0,0015781	0,0015833	0,0015796
0,0015337	0,0015337	0,0015337	0,0015335	0,0015326	0,0015372	0,0015337	0,0015292	0,0015290
0,0001576	0,0001576	0,0001575	0,0001319	0,0006001	0,0004550	0,0001575	0,0005991	0,0005270
0,0001558	0,0001558	0,0001558	0,0001312	0,0006020	0,0004546	0,0001558	0,0006003	0,0005280
0,0006104	0,0006104	0,0006091	0,0006001	0,0001470	0,0006013	0,0006091	0,0005960	0,0005952
0,0006039	0,0006039	0,0006031	0,0005925	0,0005949	0,0006354	0,0006031	0,0000709	0,0005664
0,0005856	0,0005856	0,0005849	0,0005698	0,0006138	0,0005868	0,0005849	0,0005896	0,0005763
0,0005335	0,0005335	0,0005320	0,0005188	0,0005833	0,0005413	0,0005320	0,0005649	0,0000637
0,0009659	0,0009659	0,0009671	0,0009590	0,0010137	0,0009705	0,0009671	0,0009791	0,0009771
0,0009780	0,0009780	0,0009793	0,0009709	0,0009638	0,0009732	0,0009793	0,0009664	0,0009808
0,0009623	0,0009623	0,0009629	0,0009558	0,0010016	0,0009769	0,0009629	0,0009772	0,0009736
0,0006019	0,0006019	0,0006012	0,0005910	0,0005946	0,0006359	0,0006012	0,0000000	0,0005664
0,0001663	0,0001663	0,0001661	0,0001099	0,0005981	0,0004511	0,0001661	0,0005953	0,0005283
0,0006967	0,0006967	0,0006974	0,0006838	0,0007120	0,0007226	0,0006974	0,0007182	0,0007095
0,0006526	0,0006526	0,0006542	0,0006413	0,0007094	0,0006372	0,0006542	0,0006995	0,0006417
0,0002030	0,0002030	0,0002015	0,0001585	0,0006085	0,0004620	0,0002015	0,0006019	0,0005407
0,0006191	0,0006191	0,0006198	0,0006026	0,0006643	0,0006087	0,0006198	0,0006544	0,0005868
0,0001712	0,0001712	0,0001700	0,0001079	0,0005998	0,0004486	0,0001700	0,0005964	0,0005245
0,0005988	0,0005988	0,0005989	0,0005871	0,0006432	0,0006096	0,0005989	0,0006702	0,0005922
0,0009228	0,0009228	0,0009237	0,0009162	0,0009429	0,0009294	0,0009237	0,0009386	0,0009342
0,0006038	0,0006038	0,0006019	0,0005925	0,0001431	0,0006041	0,0006019	0,0005950	0,0005968
0,0000000	0,0000000	0,0000355	0,0001469	0,0006050	0,0004633	0,0000355	0,0006019	0,0005374
0,0000438	0,0000438	0,0000260	0,0001485	0,0006037	0,0004643	0,0000260	0,0006013	0,0005367

0,0006081	0,0006081	0,0006061	0,0005962	0,0001162	0,0006047	0,0006061	0,0005990	0,0005918
0,0000000	0,0000000	0,0000355	0,0001469	0,0006050	0,0004633	0,0000355	0,0006019	0,0005374
	0,0000000	0,0000355	0,0001469	0,0006050	0,0004633	0,0000355	0,0006019	0,0005374
0,0000000		0,0000355	0,0001469	0,0006050	0,0004633	0,0000355	0,0006019	0,0005374
0,0000497	0,0000497		0,0001464	0,0006031	0,0004630	0,0000000	0,0006012	0,0005358
0,0008945	0,0008945	0,0008945		0,0005945	0,0004436	0,0001464	0,0005909	0,0005225
0,0150856	0,0150856	0,0150856	0,0143783		0,0006018	0,0006031	0,0005945	0,0005888
0,0089947	0,0089947	0,0089947	0,0082924	0,0153965		0,0004630	0,0006359	0,0005403
0,0000497	0,0000497	0,0000000	0,0008945	0,0150856	0,0089947		0,0006012	0,0005358
0,0150111	0,0150111	0,0150111	0,0143286	0,0141981	0,0161166	0,0150111		0,0005664
0,0117527	0,0117527	0,0117527	0,0110499	0,0147033	0,0126391	0,0117527	0,0132131	
0,0138986	0,0138986	0,0138986	0,0132664	0,0161996	0,0135397	0,0138986	0,0161499	0,0121006
0,0010436	0,0010436	0,0010436	0,0007451	0,0149298	0,0088416	0,0010436	0,0148553	0,0115753
0,0010933	0,0010933	0,0010933	0,0004221	0,0146018	0,0085158	0,0010933	0,0145521	0,0112733
0,0114627	0,0114627	0,0114627	0,0107548	0,0150002	0,0121023	0,0114627	0,0131283	0,0057900
0,0149820	0,0149820	0,0149820	0,0142715	0,0006484	0,0152695	0,0149820	0,0141653	0,0146479
0,0390325	0,0390325	0,0390325	0,0385037	0,0405685	0,0404450	0,0390325	0,0395480	0,0398536
0,0020241	0,0020241	0,0020241	0,0013652	0,0146126	0,0093791	0,0020241	0,0146884	0,0117573
0,0019744	0,0019744	0,0019744	0,0012646	0,0145437	0,0093077	0,0019744	0,0146196	0,0116617
0,0020441	0,0020441	0,0020441	0,0013364	0,0149276	0,0092539	0,0020441	0,0148268	0,0117519
0,0391331	0,0391331	0,0391331	0,0386042	0,0406690	0,0405456	0,0391331	0,0396237	0,0399542
0,0391581	0,0391581	0,0391581	0,0386291	0,0406939	0,0405705	0,0391581	0,0396486	0,0399791
0,0391102	0,0391102	0,0391102	0,0385812	0,0406462	0,0405227	0,0391102	0,0396008	0,0399313

wCer1	wSan	wAu_KB177	wCobs-BR2009-alpha	wCobs-JP2010-OypB	wLeh*	wPro*
0,0008753	0,0008738	0,0008782	0,0008858	0,0008579	0,0008711	0,0008693
0,0006268	0,0006027	0,0005957	0,0005708	0,0005965	0,0009771	0,0006100
0,0005780	0,0000417	0,0001483	0,0005370	0,0005962	0,0009620	0,0002151
0,0006970	0,0006899	0,0006862	0,0007474	0,0007093	0,0009953	0,0006929
0,0005783	0,0000244	0,0001462	0,0005377	0,0005966	0,0009617	0,0002135
0,0006281	0,0006033	0,0005965	0,0005727	0,0005964	0,0009781	0,0006099
0,0015803	0,0015703	0,0015712	0,0015590	0,0015620	0,0015880	0,0015678
0,0006971	0,0006567	0,0006438	0,0007077	0,0007170	0,0010002	0,0006620
0,0006253	0,0006008	0,0005942	0,0005698	0,0005939	0,0009753	0,0006089
0,0015656	0,0015389	0,0015415	0,0015412	0,0015373	0,0015753	0,0015464
0,0006085	0,0006006	0,0005954	0,0006063	0,0001215	0,0009989	0,0006117
0,0005867	0,0001583	0,0001645	0,0005428	0,0006015	0,0009596	0,0002245
0,0006693	0,0006462	0,0006503	0,0006944	0,0006936	0,0010091	0,0006574
0,0005841	0,0001969	0,0001759	0,0005479	0,0006067	0,0009575	0,0002363
0,0007072	0,0006967	0,0006955	0,0007359	0,0007563	0,0009716	0,0006901
0,0007056	0,0006959	0,0006943	0,0007351	0,0007534	0,0009701	0,0006881
0,0007023	0,0006992	0,0006976	0,0007355	0,0007492	0,0009754	0,0006917
0,0006836	0,0006791	0,0006779	0,0007179	0,0007315	0,0009633	0,0006715
0,0005942	0,0002168	0,0001951	0,0005499	0,0006052	0,0009746	0,0002451
0,0006527	0,0005929	0,0005875	0,0006715	0,0006653	0,0009460	0,0006016
0,0006692	0,0006151	0,0006087	0,0006829	0,0006816	0,0009578	0,0006207
0,0006260	0,0006011	0,0005941	0,0005697	0,0005931	0,0009735	0,0006088
0,0006268	0,0006027	0,0005957	0,0005708	0,0005965	0,0009769	0,0006100
0,0006268	0,0006027	0,0005957	0,0005708	0,0005965	0,0009769	0,0006100
0,0006313	0,0006043	0,0005974	0,0005749	0,0005995	0,0009826	0,0006119
0,0016049	0,0015782	0,0015801	0,0015894	0,0015908	0,0016189	0,0015873
0,0015618	0,0015326	0,0015356	0,0015368	0,0015325	0,0015702	0,0015413
0,0005775	0,0000428	0,0001458	0,0005365	0,0005950	0,0009605	0,0002139
0,0005783	0,0000244	0,0001462	0,0005377	0,0005966	0,0009614	0,0002135
0,0006092	0,0006069	0,0006020	0,0006029	0,0001320	0,0010039	0,0006153
0,0006268	0,0006027	0,0005957	0,0005708	0,0005965	0,0009769	0,0006100
0,0006056	0,0005798	0,0005709	0,0005960	0,0006141	0,0009648	0,0005831
0,0005416	0,0005246	0,0005242	0,0003744	0,0005879	0,0009696	0,0005470
0,0009845	0,0009611	0,0009644	0,0009842	0,0010124	0,0004604	0,0009454
0,0009672	0,0009757	0,0009775	0,0009888	0,0009591	0,0007222	0,0009534
0,0009658	0,0009649	0,0009602	0,0009950	0,0009990	0,0001297	0,0009348
0,0006249	0,0006008	0,0005941	0,0005689	0,0005964	0,0009728	0,0006093
0,0005779	0,0001507	0,0000359	0,0005332	0,0005931	0,0009573	0,0002081
0,0006968	0,0006887	0,0006856	0,0007472	0,0007098	0,0010002	0,0006916
0,0006508	0,0006505	0,0006383	0,0006474	0,0007075	0,0009818	0,0006507
0,0005862	0,0001962	0,0001739	0,0005410	0,0006038	0,0009653	0,0002292
0,0005679	0,0006162	0,0006026	0,0005976	0,0006659	0,0009997	0,0006241
0,0005750	0,0001517	0,0001227	0,0005342	0,0005938	0,0009533	0,0002046
0,0006158	0,0005942	0,0005927	0,0006327	0,0006442	0,0009904	0,0006042
0,0009371	0,0009200	0,0009255	0,0009324	0,0009422	0,0006805	0,0009005
0,0006076	0,0006004	0,0005957	0,0006052	0,0001224	0,0009955	0,0006111
0,0005867	0,0001583	0,0001645	0,0005428	0,0006015	0,0009596	0,0002245
0,0005859	0,0001607	0,0001661	0,0005423	0,0006001	0,0009610	0,0002250

0,0006120	0,0006039	0,0005993	0,0006007	0,0001417	0,0010004	0,0006134
0,0005867	0,0001583	0,0001645	0,0005428	0,0006015	0,0009596	0,0002245
0,0005867	0,0001583	0,0001645	0,0005428	0,0006015	0,0009596	0,0002245
0,0005867	0,0001583	0,0001645	0,0005428	0,0006015	0,0009596	0,0002245
0,0005855	0,0001586	0,0001643	0,0005419	0,0005997	0,0009603	0,0002236
0,0005726	0,0001330	0,0001042	0,0005280	0,0005895	0,0009529	0,0001889
0,0006077	0,0006020	0,0005975	0,0005999	0,0001225	0,0010002	0,0006118
0,0005605	0,0004540	0,0004506	0,0005497	0,0005993	0,0009745	0,0004768
0,0005855	0,0001586	0,0001643	0,0005419	0,0005997	0,0009603	0,0002236
0,0006248	0,0006007	0,0005940	0,0005689	0,0005963	0,0009727	0,0006093
0,0005431	0,0005280	0,0005275	0,0003729	0,0005932	0,0009722	0,0005495
	0,0005786	0,0005749	0,0005695	0,0006085	0,0009608	0,0005848
0,0137681		0,0001474	0,0005389	0,0005964	0,0009622	0,0002139
0,0134900	0,0009438		0,0005320	0,0005925	0,0009573	0,0002034
0,0130590	0,0112828	0,0109794		0,0006006	0,0009923	0,0005579
0,0161262	0,0148255	0,0144960	0,0149710		0,0009959	0,0006066
0,0404652	0,0389154	0,0386779	0,0398261	0,0405851		0,0009299
0,0139367	0,0019220	0,0015927	0,0114187	0,0145303	0,0348784	
0,0138421	0,0017711	0,0014923	0,0113980	0,0144611	0,0348787	0,0009625
0,0140518	0,0018918	0,0015633	0,0115405	0,0148471	0,0354794	0,0008599
0,0405409	0,0390160	0,0387784	0,0399270	0,0407112	0,0006214	0,0349804
0,0405658	0,0390409	0,0388033	0,0399520	0,0407362	0,0006462	0,0350057
0,0405180	0,0389931	0,0387554	0,0399041	0,0406882	0,0006214	0,0349569

wPsd*	wSpt*	wStv_south*	wStv_central*	wStv_north*
0,0008611	0,0008699	0,0008693	0,0008682	0,0008694
0,0006085	0,0006146	0,0009824	0,0009816	0,0009822
0,0002073	0,0002139	0,0009655	0,0009646	0,0009649
0,0006847	0,0006960	0,0009958	0,0009951	0,0009956
0,0002076	0,0002124	0,0009652	0,0009643	0,0009645
0,0006089	0,0006151	0,0009832	0,0009824	0,0009831
0,0015687	0,0015685	0,0015895	0,0015891	0,0015896
0,0006449	0,0006532	0,0010033	0,0010032	0,0010032
0,0006074	0,0006140	0,0009799	0,0009791	0,0009798
0,0015438	0,0015434	0,0015773	0,0015769	0,0015773
0,0006061	0,0006150	0,0010006	0,0010003	0,0010002
0,0002264	0,0002296	0,0009631	0,0009622	0,0009624
0,0006526	0,0006665	0,0010123	0,0010112	0,0010123
0,0002312	0,0002385	0,0009607	0,0009600	0,0009598
0,0006894	0,0006942	0,0009732	0,0009730	0,0009733
0,0006870	0,0006920	0,0009718	0,0009716	0,0009719
0,0006903	0,0006963	0,0009774	0,0009772	0,0009776
0,0006683	0,0006739	0,0009649	0,0009649	0,0009650
0,0002405	0,0002425	0,0009791	0,0009784	0,0009786
0,0005873	0,0005918	0,0009507	0,0009507	0,0009502
0,0006060	0,0006130	0,0009621	0,0009623	0,0009615
0,0006070	0,0006136	0,0009782	0,0009774	0,0009781
0,0006085	0,0006146	0,0009822	0,0009814	0,0009820
0,0006085	0,0006146	0,0009822	0,0009814	0,0009820
0,0006108	0,0006168	0,0009878	0,0009870	0,0009876
0,0015860	0,0015850	0,0016213	0,0016207	0,0016212
0,0015376	0,0015384	0,0015722	0,0015717	0,0015721
0,0002065	0,0002133	0,0009639	0,0009631	0,0009633
0,0002076	0,0002124	0,0009648	0,0009640	0,0009642
0,0006099	0,0006208	0,0010058	0,0010053	0,0010053
0,0006085	0,0006146	0,0009822	0,0009814	0,0009820
0,0005787	0,0005931	0,0009698	0,0009692	0,0009689
0,0005378	0,0005378	0,0009716	0,0009710	0,0009714
0,0009351	0,0009374	0,0004612	0,0004627	0,0004613
0,0009509	0,0009496	0,0007246	0,0007242	0,0007246
0,0009249	0,0009303	0,0000243	0,0000344	0,0000000
0,0006070	0,0006141	0,0009775	0,0009768	0,0009774
0,0001972	0,0002013	0,0009610	0,0009601	0,0009604
0,0006853	0,0006944	0,0010002	0,0009993	0,0009998
0,0006454	0,0006529	0,0009850	0,0009841	0,0009849
0,0002240	0,0002315	0,0009689	0,0009682	0,0009684
0,0006231	0,0006268	0,0010041	0,0010031	0,0010036
0,0001937	0,0001999	0,0009561	0,0009555	0,0009555
0,0005961	0,0006146	0,0009937	0,0009931	0,0009935
0,0008912	0,0008926	0,0006820	0,0006828	0,0006826
0,0006053	0,0006141	0,0009973	0,0009970	0,0009969
0,0002264	0,0002296	0,0009631	0,0009622	0,0009624
0,0002267	0,0002318	0,0009643	0,0009635	0,0009636

0,0006069	0,0006166	0,0010016	0,0010012	0,0010013
0,0002264	0,0002296	0,0009631	0,0009622	0,0009624
0,0002264	0,0002296	0,0009631	0,0009622	0,0009624
0,0002264	0,0002296	0,0009631	0,0009622	0,0009624
0,0002246	0,0002290	0,0009637	0,0009629	0,0009630
0,0001800	0,0001890	0,0009566	0,0009558	0,0009559
0,0006048	0,0006150	0,0010021	0,0010016	0,0010017
0,0004702	0,0004678	0,0009779	0,0009773	0,0009770
0,0002246	0,0002290	0,0009637	0,0009629	0,0009630
0,0006070	0,0006141	0,0009775	0,0009767	0,0009773
0,0005398	0,0005407	0,0009740	0,0009734	0,0009737
0,0005849	0,0005882	0,0009664	0,0009655	0,0009658
0,0002077	0,0002133	0,0009657	0,0009649	0,0009650
0,0001930	0,0001976	0,0009609	0,0009601	0,0009603
0,0005490	0,0005558	0,0009961	0,0009952	0,0009951
0,0006017	0,0006117	0,0009995	0,0009991	0,0009990
0,0009204	0,0009267	0,0001270	0,0001291	0,0001270
0,0001626	0,0001574	0,0009357	0,0009345	0,0009349
	0,0001650	0,0009260	0,0009249	0,0009250
0,0010626		0,0009312	0,0009301	0,0009304
0,0349808	0,0355811		0,0000000	0,0000000
0,0350061	0,0356063	0,0000000		0,0000239
0,0349573	0,0355577	0,0000000	0,0000249	

Apêndice E - Estimates of divergence time among host species.

	<i>Anoplolepis gracilipes</i>	<i>Cardiocondyla obscurior</i>	<i>Apterostigma dentigerum</i>
<i>Anoplolepis gracilipes</i>			
<i>Cardiocondyla obscurior</i>	22.992.066		
<i>Apterostigma dentigerum</i>	23.755.583	23.358.760	
<i>Nomada flava</i>	27.539.076	27.881.186	26.568.438
<i>Carposina sasakii</i>	28.584.792	28.244.906	28.213.652
<i>Culex quinquefasciatus</i>	28.999.193	29.289.411	29.533.422
<i>Aedes albopictus</i>	29.048.322	29.271.096	29.246.867
<i>Glossina morsitans</i>	28.921.023	28.599.402	28.551.170
<i>Ceratitis capitata</i>	29.033.744	29.278.626	29.406.146
<i>Rhagoletis cerasi</i>	29.113.588	29.569.393	29.648.174
<i>Drosophila borealis</i>	29.470.074	29.829.813	29.896.942
<i>Drosophila bifasciata</i>	28.929.734	29.472.970	29.095.331
<i>Drosophila incompta</i>	29.058.621	29.210.533	29.179.760
<i>Drosophila triauraria</i>	28.959.451	29.577.616	29.246.948
<i>Drosophila suzukii</i>	29.078.320	29.338.424	28.920.971
<i>Drosophila baimaii</i>	26.331.114	26.418.968	26.095.212
<i>Drosophila santomea</i>	28.848.011	29.330.949	28.731.450
<i>Drosophila teissieri</i>	28.892.587	29.345.899	28.731.450
<i>Drosophila yakuba</i>	28.877.728	29.345.899	28.776.935
<i>Drosophila melanogaster</i>	28.581.026	29.182.094	28.534.814
<i>Drosophila mauritiana</i>	28.920.203	29.186.865	28.744.435
<i>Drosophila simulans</i>	29.016.802	29.261.626	28.850.587
<i>Drosophila sechellia</i>	29.048.603	29.248.727	28.958.875
<i>Drosophila ananassae</i>	28.647.420	29.398.222	28.776.935
<i>Drosophila auraria</i>	28.729.142	29.487.919	28.814.839
<i>Drosophila lehrmanae*</i>	28.384.999	28.730.781	28.387.892
<i>Drosophila sturtevantii central*</i>	28.204.059	28.601.909	28.195.575
<i>Drosophila sturtevantii south*</i>	28.175.145	28.550.501	28.158.398
<i>Drosophila sturtevantii north*</i>	28.144.267	28.504.806	28.119.190
<i>Drosophila prosaltans*</i>	28.159.766	28.392.868	27.965.412
<i>Drosophila septentriosaltans*</i>	28.294.837	28.451.897	28.020.192
<i>Drosophila pseudosaltans*</i>	28.250.674	28.514.285	28.104.666
<i>Haematobia irritans</i>	28.813.000	28.943.409	28.778.004
<i>Nasonia vitripennis</i>	28.113.862	28.208.468	27.331.752
<i>Wiebesia pumilae</i>	30.274.166	30.309.438	29.455.229
<i>Kradibia gibbosae</i>	29.118.086	29.014.990	27.961.194
<i>Ceratosolen solmsi</i>	30.475.257	30.887.911	30.278.693
<i>Drosophila bicornuta</i>	23.004.191	22.705.919	23.260.155
<i>Drosophila nikananu</i>	23.790.323	22.983.871	22.899.243
<i>Drosophila subpulchrella</i>	28.205.093	26.603.514	27.155.295
<i>Drosophila tropicalis</i>	25.046.666	24.426.880	24.175.178
<i>Drosophila arawakana</i>	23.618.232	24.476.160	23.608.426
<i>Drosophila neotestacea</i>	25.745.657	25.653.544	25.474.220
<i>Drosophila recens</i>	23.750.171	24.308.269	24.430.373
<i>Rhagoletis cingulata</i>	23.504.059	23.420.315	23.301.853
<i>Drosophila orientacea</i>	24.903.801	25.173.517	24.636.554
<i>Nasonia oneida</i>	19.884.023	19.884.023	19.704.434
<i>Muscidifurax uniraptor</i>	18.485.997	17.561.697	15.250.947
<i>Nomada ferruginata</i>	21.080.498	23.041.475	22.285.151
<i>Nomada leucophthalma</i>	20.957.937	23.286.597	22.162.029
<i>Nomada panzeri</i>	21.154.745	22.557.270	21.481.899
<i>Dactylopius coccus</i>	23.074.111	26.044.046	25.587.133

<i>Nomada flava</i>	<i>Carposina sasakii</i>	<i>Culex quinquefasciatus</i>	<i>Aedes albopictus</i>	<i>Glossina morsitans</i>
26.997.357				
27.956.578	20.516.094			
27.808.676	20.596.505	10.761.981		
27.843.516	20.331.299	17.333.965	16.924.346	
28.094.989	20.421.791	17.175.858	17.437.979	15.280.685
28.141.721	20.424.128	17.766.908	18.158.367	16.331.212
28.616.025	20.880.550	17.875.525	17.946.367	16.045.588
27.979.562	20.164.959	17.197.850	17.481.903	15.271.993
27.767.534	20.216.770	16.408.291	17.086.652	15.161.430
28.002.647	19.820.071	16.633.385	17.203.715	14.993.846
27.748.707	19.710.000	16.831.314	17.013.376	14.933.001
24.066.806	17.173.137	14.806.659	15.291.160	13.608.661
27.548.633	19.585.253	16.787.330	17.086.584	14.811.312
27.579.414	19.599.930	16.794.661	17.042.659	14.793.928
27.579.414	19.592.592	16.809.322	17.115.867	14.820.004
27.425.667	19.478.728	16.652.588	16.996.043	14.616.120
27.384.566	19.684.230	16.577.753	17.155.585	14.715.699
27.376.870	19.618.176	16.739.058	17.053.076	14.663.547
27.594.804	19.732.014	16.779.999	16.954.811	14.733.083
27.656.365	19.849.423	16.428.125	16.661.981	14.767.852
27.733.317	19.900.790	16.472.110	16.691.264	14.767.852
26.718.754	18.910.920	16.171.901	16.253.034	14.236.662
26.451.862	18.767.090	15.862.681	15.966.486	13.937.368
26.433.389	18.754.689	15.847.626	15.951.546	13.905.551
26.386.025	18.682.363	15.808.706	15.935.028	13.859.099
26.473.279	18.903.423	15.846.067	16.179.593	13.961.369
26.599.300	19.021.973	15.915.834	16.306.437	14.176.765
26.619.371	19.050.624	16.005.626	16.212.172	14.162.291
27.256.325	19.452.653	15.954.371	16.130.498	12.679.893
26.171.111	26.685.158	27.566.145	26.986.115	26.981.751
28.242.195	28.526.261	28.845.373	28.835.115	28.715.641
25.401.826	26.701.910	26.944.178	26.637.228	27.259.962
28.272.208	29.154.308	29.248.133	29.369.094	29.262.990
21.273.573	14.297.203	13.366.396	14.818.455	11.520.737
20.973.650	14.992.669	14.057.078	15.008.859	12.059.668
26.025.077	18.780.847	17.735.101	17.743.298	12.954.474
22.334.812	15.582.903	14.361.965	14.361.965	13.160.842
22.539.941	15.997.820	14.255.203	13.852.229	12.829.912
23.896.581	16.304.047	14.942.804	14.437.047	13.673.655
23.023.648	16.556.907	14.865.468	14.803.528	14.300.450
21.453.955	15.213.435	15.030.340	14.639.941	12.908.082
23.041.475	16.542.597	15.572.549	14.719.874	13.550.646
17.507.447	17.824.323	17.745.104	19.091.831	15.868.887
14.557.723	15.482.022	17.099.547	15.713.097	17.482.517
490.244	20.957.937	19.732.327	20.222.571 n/c	
490.244	21.203.059	19.732.327	20.590.254 n/c	
1.051.893	20.570.360	19.167.835	19.752.221 n/c	
24.216.394	24.901.764	25.587.133	23.302.568	24.931.312

Ceratitis capitata *Rhagoletis cerasi* *Drosophila borealis* *Drosophila bifasciata* *Drosophila incompta*

11.642.229				
14.753.859	15.845.990			
13.858.944	15.410.557	10.877.039		
14.081.018	15.291.084	9.841.968	10.276.932	
13.975.959	15.219.941	10.964.816	9.361.187	10.284.304
13.719.989	14.831.378	10.591.764	9.112.530	9.782.990
12.107.248	13.105.537	10.305.823	8.630.080	9.174.696
13.617.601	14.780.059	10.518.616	8.732.232	9.591.311
13.581.034	14.721.408	10.533.246	8.702.978	9.524.961
13.639.542	14.802.053	10.577.134	8.761.486	9.628.173
13.738.354	15.010.060	10.858.049	9.129.641	9.992.154
13.678.590	14.951.399	10.783.903	8.792.334	9.851.142
13.846.829	15.010.060	10.608.318	8.865.482	9.902.757
13.895.512	15.087.977	10.840.465	9.046.709	10.070.508
13.573.721	14.552.786	10.738.059	9.141.784	9.834.596
13.595.661	14.611.437	10.738.059	9.112.530	9.827.224
13.309.364	14.244.109	10.737.938	9.438.219	9.752.646
13.160.381	14.066.103	10.608.486	9.137.718	9.509.551
13.142.886	14.034.584	10.566.482	9.101.762	9.510.886
13.056.202	13.941.416	10.513.466	9.069.067	9.448.624
12.747.522	14.224.052	10.297.973	9.040.638	9.623.796
13.049.451	14.303.356	10.488.746	9.489.838	9.872.183
13.000.825	14.341.709	10.466.365	9.285.244	9.787.179
13.478.687	14.223.728	14.323.248	13.617.794	13.181.318
27.517.386	27.377.087	28.133.419	27.562.082	27.439.931
28.985.725	29.374.763	29.449.034	29.060.778	28.755.899
27.275.501	27.513.791	27.729.252	27.006.334	26.823.937
29.731.351	29.986.472	29.991.106	29.478.319	29.320.562
9.717.630	12.584.517	10.536.741	8.563.429	8.898.520
10.323.166	12.812.441	10.469.594	8.090.141	8.822.280
13.480.576	13.497.669	11.924.718	9.633.846	10.391.540
10.835.103	12.871.230	9.926.118	8.944.414	8.871.695
11.031.412	13.298.140	9.923.233	9.923.233	8.714.312
11.954.243	13.701.401	11.632.398	7.172.546	9.701.328
12.325.950	13.193.102	10.684.555	10.158.069	9.848.372
9.508.990	8.672.422	13.106.233	12.353.322	11.600.410
12.072.091	13.373.544	11.129.661	9.424.309	10.052.597
19.408.708	19.329.489	20.834.654	18.616.516	18.774.954
20.334.597	17.099.547	19.872.447	17.561.697	18.948.147
19.609.766	21.203.059	21.938.425	21.693.303	18.506.716
19.609.766	20.957.937	21.938.425	21.815.864	18.629.277
18.934.081	20.570.360	21.037.868	21.154.745	18.466.573
27.414.786	25.358.677	27.186.329	25.815.590	26.729.416

Drosophila triauraria *Drosophila suzukii* *Drosophila baimaii* *Drosophila santomea* *Drosophila teissieri*

7.598.651				
5.920.961	7.149.839			
7.869.248	6.011.637	7.177.768		
7.913.128	6.004.324	7.149.839	285.224	
7.891.188	6.048.204	7.163.804	138.955	277.910
8.068.905	6.532.668	7.443.095	6.005.958	6.035.219
8.177.895	6.363.836	7.052.088	5.903.006	5.895.692
8.148.636	6.356.521	7.275.520	5.954.210	5.961.524
8.454.322	6.311.488	7.415.165	5.989.697	5.982.383
8.088.650	7.818.054	7.373.272	7.949.695	7.949.695
7.935.068	7.752.233	7.373.272	7.913.128	7.913.128
9.423.472	9.290.747	8.476.470	9.106.407	9.091.660
9.344.387	9.049.146	8.474.244	8.827.715	8.812.953
9.330.784	9.027.884	8.453.606	8.798.863	8.784.087
9.246.602	8.958.108	8.361.438	8.721.395	8.706.600
9.144.638	8.958.923	8.322.860	8.750.921	8.713.778
9.452.913	9.172.279	8.630.080	9.024.578	8.980.267
9.314.909	8.996.007	8.575.706	8.951.509	8.884.762
12.951.547	12.651.370	10.962.156	12.651.370	12.651.370
27.353.504	27.204.520	24.983.307	27.137.477	27.122.578
28.888.155	28.865.639	26.483.438	28.587.940	28.602.951
26.826.890	26.849.321	23.976.346	26.819.413	26.834.367
29.451.208	29.270.470	27.359.549	29.044.548	29.116.843
6.701.814	7.781.551	5.547.613	7.632.621	7.632.621
6.113.364	7.358.002	5.161.583	7.394.609	7.358.002
8.816.841	817.005	7.923.414	6.399.869	6.331.786
9.162.570	9.235.289	9.140.068	8.726.257	8.689.898
9.520.260	9.066.914	8.412.081	8.815.055	8.865.427
9.793.284	9.149.594	9.839.261	9.471.439	9.471.439
9.755.463	9.105.099	8.826.371	9.693.524	9.755.463
11.935.038	11.823.495	11.126.355	11.488.868	11.377.326
10.815.517	9.065.288	9.783.331	9.738.453	9.783.331
19.329.489	18.695.735	19.329.489	17.745.104	17.982.762
18.717.072	17.330.622	17.099.547	17.561.697	17.792.772
20.957.937	20.835.376	20.100.010	18.751.838	18.506.716
21.080.498	20.957.937	20.222.571	18.629.277	18.384.155
20.687.237	20.453.483	19.869.098	18.466.573	18.232.819
26.272.503	23.074.111	25.358.677	26.500.960	26.272.503

Drosophila yakuba *Drosophila melanogaster* *Drosophila mauritiana* *Drosophila simulans* *Drosophila sechellia*

6.049.850				
5.968.839	3.797.391			
6.012.728	3.907.142	2.662.570		
6.055.518	4.060.057	2.903.957	2.823.495	
7.986.262	8.229.845	8.024.285	8.016.970	8.242.232
7.949.695	8.178.637	7.987.711	7.980.396	8.205.665
9.150.649	9.300.672	9.078.573	9.270.322	9.452.967
8.864.620	9.081.163	8.844.096	9.050.803	9.159.861
8.835.802	9.045.146	8.807.864	9.029.539	9.116.538
8.758.381	8.967.973	8.745.191	8.959.751	9.046.875
8.765.778	8.805.355	8.574.214	8.700.524	8.832.636
9.039.348	9.130.477	8.944.980	9.063.163	9.223.975
8.966.342	9.028.163	8.856.726	8.967.991	9.151.750
12.687.977	12.889.165	12.741.540	12.873.349	12.900.297
27.144.926	26.899.107	27.149.942	27.023.282	27.010.840
28.632.973	28.738.566	28.765.918	28.840.986	28.948.198
26.856.798	26.654.860	26.682.301	26.592.562	26.789.506
29.080.696	29.235.114	29.195.682	29.105.292	29.225.286
7.707.086	7.818.783	8.191.106	8.079.409	8.116.641
7.467.822	7.577.643	7.907.106	7.577.643	7.980.320
6.467.953	6.672.204	6.672.204	6.501.995	6.808.372
8.689.898	9.380.727	9.344.367	9.598.883	9.344.367
8.865.427	8.966.170	9.570.631	9.066.914	9.268.401
9.517.416	9.563.394	9.471.439	9.287.527	9.379.483
9.755.463	9.352.857	9.507.705	9.383.826	9.538.675
11.572.525	11.711.953	11.990.809	11.600.410	11.349.440
9.828.208	9.379.432	9.469.187	9.424.309	9.334.554
17.745.104	17.428.227	18.061.981	18.695.735	18.537.296
17.561.697	15.944.172	17.099.547	18.254.922	17.099.547
18.751.838	19.364.644	19.487.205	20.345.132	19.364.644
18.629.277	19.487.205	19.732.327	20.712.815	19.732.327
18.466.573	19.284.712	19.401.590	20.219.729	19.401.590
26.500.960	26.272.503	27.186.329	26.957.873	26.500.960

Drosophila ananassae *Drosophila auraria* *Drosophila lehrmanae** *Drosophila sturtevantii central**

153.582			
8.973.682	8.936.814		
8.680.094	8.665.332	1.933.831	
8.643.719	8.628.944	1.898.663	0
8.603.038	8.588.244	1.834.526	44.424
8.646.920	8.654.349	5.772.042	5.420.461
8.958.112	8.965.497	6.055.772	5.795.747
8.892.178	8.884.762	5.881.149	5.612.422
12.622.084	12.673.334	12.393.888	12.059.090
27.264.113	27.323.707	26.516.153	26.144.823
28.760.564	28.783.080	27.868.182	27.760.604
26.625.016	26.684.830	25.981.977	25.696.872
29.469.282	29.487.356	28.758.591	28.784.397
8.153.874	8.153.874	9.568.701	9.358.269
7.870.499	7.870.499	8.822.280	8.651.026
8.987.050	8.987.050	10.791.269	10.348.725
7.853.632	7.853.632	8.108.147	7.311.537
9.016.542	9.016.542	8.462.453	8.568.548
9.655.350	9.655.350	9.195.571	8.704.703
9.538.675	9.538.675	9.228.978	9.401.879
11.684.067	11.684.067	11.070.584	11.109.995
9.873.086	9.873.086	9.558.942	9.225.590
19.091.831	19.091.831	18.854.173	18.793.415
16.406.322	16.406.322	18.948.147	17.612.162
20.835.376	20.835.376	19.854.888	19.609.766
21.203.059	21.203.059	19.732.327	19.487.205
20.336.606	20.336.606	19.167.835	19.050.958
26.044.046	26.044.046	25.815.590	26.347.141

*Drosophila sturtevantii south***Drosophila sturtevantii north***Drosophila prosaltans***Drosophila septentriosaltans**

37.051			
5.366.420	5.313.742		
5.734.471	5.689.985	1.664.165	
5.565.579	5.498.365	1.835.378	2.158.350
12.033.169	11.996.801	12.055.128	12.420.597
26.121.627	26.080.443	26.130.454	26.309.929
27.756.375	27.679.060	27.771.086	27.912.426
25.652.957	25.636.099	25.506.871	25.645.750
28.774.034	28.737.717	28.605.141	28.837.413
9.320.985	9.353.944	8.377.267	8.675.126
8.651.026	8.683.736	8.236.569	8.492.818
10.298.029	10.187.477	9.255.508	9.802.174
7.288.311	7.255.148	7.017.365	7.162.803
8.528.806	8.573.907	8.361.709	8.815.055
8.627.371	8.745.765	8.827.749	9.103.616
9.343.416	9.367.246	8.702.492	9.198.008
11.033.889	11.053.032	11.321.554	11.377.326
9.265.423	9.215.306	9.424.309	9.424.309
18.811.913	18.793.415	19.725.585	19.725.585
17.792.772	17.612.162	18.485.997	18.254.922
19.546.617	19.487.205	19.487.205	19.732.327
19.423.682	19.364.644	19.487.205	19.732.327
18.989.122	18.934.081	19.284.712	19.284.712
26.272.503	26.272.503	25.130.220	25.130.220

*Drosophila pseudosaltans** *Haematobia irritans* *Nasonia vitripennis* *Wiebesia pumilae* *Kradibia gibbosae*

12.257.886				
26.222.324	26.772.405			
27.904.215	28.123.301	24.854.574		
25.612.594	26.235.861	22.369.280	21.964.609	
28.732.285	28.801.198	25.974.948	24.967.910	22.680.030
8.675.126	9.196.378	21.587.974	23.807.381	21.163.268
8.529.425	9.627.634	21.352.681	23.853.834	21.303.886
9.465.067	12.713.713	24.685.879	25.446.460	23.580.659
7.090.084	9.853.399	21.206.962	24.156.724	21.771.266
8.815.055	10.124.720	21.896.693	25.287.473	22.932.514
8.965.682	10.207.084	24.602.820	27.937.123	22.895.810
9.105.099	10.994.252	23.750.375	26.423.628	23.330.717
11.711.953	10.512.872	21.930.317	24.196.355	21.443.708
9.020.410	11.174.538	24.198.071	27.621.307	23.801.367
19.408.708	17.190.570	8.951.771	18.009.690	15.210.089
18.023.847	17.792.772	9.705.148	17.099.547	15.713.097
19.609.766	17.771.350	19.419.157	21.892.935	19.295.468
19.609.766	18.139.033	19.295.468	21.892.935	19.419.157
19.167.835	17.999.065	18.864.365	21.340.313	19.218.072
24.673.307	24.444.851	24.673.307	28.557.068	26.500.960

Ceratosolen solmsi *Drosophila bicornuta* *Drosophila nikananu* *Drosophila subpulchrella* *Drosophila tropicalis*

24.485.578				
24.839.448	5.138.057			
27.101.494	8.530.870	7.620.911		
25.063.396	8.758.593	7.951.688	9.557.526	
26.541.445	9.171.849	8.905.936	9.663.614	9.015.211
28.484.973	9.174.084	8.980.664	9.083.321	9.101.210
27.163.368	9.298.001	9.067.052	8.758.360	8.613.635
25.927.672	11.542.013	11.714.231	11.479.533	12.845.379
28.471.011	9.660.618	9.460.185 n/c		8.648.576
19.882.929	20.359.338	19.646.365	19.785.614	20.430.108
22.645.346	20.103.522	19.872.447 n/c		18.433.180
23.377.202	19.854.888	20.712.815	20.590.254	20.100.010
23.253.513	19.977.449	20.712.815	20.590.254	20.100.010
22.873.043	19.401.590	20.336.606	20.102.852	19.401.590
30.613.177	26.500.960	25.815.590 n/c		31.017.370

Drosophila arawakana *Drosophila neotestacea* *Drosophila recens* *Rhagoletis cingulata* *Drosophila orientacea*

8.584.417				
7.977.096	9.103.616			
10.872.891	12.898.404	12.789.703		
8.693.663	5.145.999	8.975.533	12.847.165	
19.372.252	21.661.969	20.280.119	18.695.735	18.304.329
16.801.075 n/c		18.485.997	17.099.547	18.145.161
20.100.010	21.735.909	20.345.132	20.467.693 n/c	
20.467.693	21.859.408	20.467.693	20.345.132 n/c	
19.985.975	22.217.312	19.985.975	19.985.975 n/c	
30.388.032 n/c		26.500.960	25.587.133	25.152.992

Nasonia oneida *Muscidifurax uniraptor* *Nomada ferruginata* *Nomada leucophthalma* *Nomada panzeri*

9.936.223				
19.443.574	n/c			
19.313.081	n/c		490.244	
19.146.886	n/c		1.103.049	612.805
25.638.635	24.544.180	n/c	n/c	n/c

Dactylopius coccus

Apêndice F - P-distances of host species based on substitution rate of all three codon positions of the 13 mitochondrial genes.

	<i>Anoplolepis gracilipes</i>	<i>Cardiocondyla obscurior</i>	<i>Apterostigma dentigerum</i>
<i>Anoplolepis gracilipes</i>		0,0043	0,0043
<i>Cardiocondyla obscurior</i>	0,2851		0,0046
<i>Apterostigma dentigerum</i>	0,2946	0,2896	
<i>Nomada flava</i>	0,3415	0,3457	0,3294
<i>Carposina sasakii</i>	0,3545	0,3502	0,3498
<i>Culex quinquefasciatus</i>	0,3596	0,3632	0,3662
<i>Aedes albopictus</i>	0,3602	0,3630	0,3627
<i>Glossina morsitans</i>	0,3586	0,3546	0,3540
<i>Ceratitis capitata</i>	0,3600	0,3631	0,3646
<i>Rhagoletis cerasi</i>	0,3610	0,3667	0,3676
<i>Drosophila borealis</i>	0,3654	0,3699	0,3707
<i>Drosophila bifasciata</i>	0,3587	0,3655	0,3608
<i>Drosophila incompta</i>	0,3603	0,3622	0,3618
<i>Drosophila triauraria</i>	0,3591	0,3668	0,3627
<i>Drosophila suzukii</i>	0,3606	0,3638	0,3586
<i>Drosophila baimaii</i>	0,3265	0,3276	0,3236
<i>Drosophila santomea</i>	0,3577	0,3637	0,3563
<i>Drosophila teissieri</i>	0,3583	0,3639	0,3563
<i>Drosophila yakuba</i>	0,3581	0,3639	0,3568
<i>Drosophila melanogaster</i>	0,3544	0,3619	0,3538
<i>Drosophila mauritiana</i>	0,3586	0,3619	0,3564
<i>Drosophila simulans</i>	0,3598	0,3628	0,3577
<i>Drosophila sechellia</i>	0,3602	0,3627	0,3591
<i>Drosophila ananassae</i>	0,3552	0,3645	0,3568
<i>Drosophila auraria</i>	0,3562	0,3657	0,3573
<i>Drosophila lehrmanae</i> *	0,3520	0,3563	0,3520
<i>Drosophila sturtevantii central</i> *	0,3497	0,3547	0,3496
<i>Drosophila sturtevantii south</i> *	0,3494	0,3540	0,3492
<i>Drosophila sturtevantii north</i> *	0,3490	0,3535	0,3487
<i>Drosophila prosaltans</i> *	0,3492	0,3521	0,3468
<i>Drosophila septentrionsaltans</i> *	0,3509	0,3528	0,3475
<i>Drosophila pseudosaltans</i> *	0,3503	0,3536	0,3485
<i>Haematobia irritans</i>	0,3573	0,3589	0,3568
<i>Nasonia vitripennis</i>	0,3486	0,3498	0,3389
<i>Wiebesia pumilae</i>	0,3754	0,3758	0,3652
<i>Kradibia gibbosae</i>	0,3611	0,3598	0,3467
<i>Ceratosolen solmsi</i>	0,3779	0,3830	0,3755
<i>Drosophila bicornuta</i>	0,2853	0,2816	0,2884
<i>Drosophila nikananu</i>	0,2950	0,2850	0,2840
<i>Drosophila subpulchrella</i>	0,3497	0,3299	0,3367
<i>Drosophila tropicalis</i>	0,3106	0,3029	0,2998
<i>Drosophila arawakana</i>	0,2929	0,3035	0,2927
<i>Drosophila neotestacea</i>	0,3192	0,3181	0,3159
<i>Drosophila recens</i>	0,2945	0,3014	0,3029
<i>Rhagoletis cingulata</i>	0,2915	0,2904	0,2889
<i>Drosophila orientacea</i>	0,3088	0,3122	0,3055
<i>Nasonia oneida</i>	0,2466	0,2466	0,2443
<i>Muscidifurax uniraptor</i>	0,2292	0,2178	0,1891
<i>Nomada ferruginata</i>	0,2614	0,2857	0,2763
<i>Nomada leucophthalma</i>	0,2599	0,2888	0,2748
<i>Nomada panzeri</i>	0,2623	0,2797	0,2664
<i>Dactylopius coccus</i>	0,2861	0,3229	0,3173

<i>Nomada flava</i>	<i>Carposina sasakii</i>	<i>Culex quinquefasciatus</i>	<i>Aedes albopictus</i>	<i>Glossina morsitans</i>
0,0047	0,0047	0,0047	0,0046	0,0048
0,0046	0,0047	0,0046	0,0047	0,0050
0,0045	0,0047	0,0047	0,0049	0,0051
	0,0046	0,0047	0,0045	0,0051
0,3348		0,0042	0,0042	0,0044
0,3467	0,2544		0,0033	0,0043
0,3448	0,2554	0,1334		0,0043
0,3453	0,2521	0,2149	0,2099	
0,3484	0,2532	0,2130	0,2162	0,1895
0,3490	0,2533	0,2203	0,2252	0,2025
0,3548	0,2589	0,2217	0,2225	0,1990
0,3469	0,2500	0,2133	0,2168	0,1894
0,3443	0,2507	0,2035	0,2119	0,1880
0,3472	0,2458	0,2063	0,2133	0,1859
0,3441	0,2444	0,2087	0,2110	0,1852
0,2984	0,2129	0,1836	0,1896	0,1687
0,3416	0,2429	0,2082	0,2119	0,1837
0,3420	0,2430	0,2083	0,2113	0,1834
0,3420	0,2429	0,2084	0,2122	0,1838
0,3401	0,2415	0,2065	0,2108	0,1812
0,3396	0,2441	0,2056	0,2127	0,1825
0,3395	0,2433	0,2076	0,2115	0,1818
0,3422	0,2447	0,2081	0,2102	0,1827
0,3429	0,2461	0,2037	0,2066	0,1831
0,3439	0,2468	0,2043	0,2070	0,1831
0,3313	0,2345	0,2005	0,2015	0,1765
0,3280	0,2327	0,1967	0,1980	0,1728
0,3278	0,2326	0,1965	0,1978	0,1724
0,3272	0,2317	0,1960	0,1976	0,1719
0,3283	0,2344	0,1965	0,2006	0,1731
0,3298	0,2359	0,1974	0,2022	0,1758
0,3301	0,2362	0,1985	0,2010	0,1756
0,3380	0,2412	0,1978	0,2000	0,1572
0,3245	0,3309	0,3418	0,3346	0,3346
0,3502	0,3537	0,3577	0,3576	0,3561
0,3150	0,3311	0,3341	0,3303	0,3380
0,3506	0,3615	0,3627	0,3642	0,3629
0,2638	0,1773	0,1657	0,1837	0,1429
0,2601	0,1859	0,1743	0,1861	0,1495
0,3227	0,2329	0,2199	0,2200	0,1606
0,2770	0,1932	0,1781	0,1781	0,1632
0,2795	0,1984	0,1768	0,1718	0,1591
0,2963	0,2022	0,1853	0,1790	0,1696
0,2855	0,2053	0,1843	0,1836	0,1773
0,2660	0,1886	0,1864	0,1815	0,1601
0,2857	0,2051	0,1931	0,1825	0,1680
0,2171	0,2210	0,2200	0,2367	0,1968
0,1805	0,1920	0,2120	0,1948	0,2168
0,0061	0,2599	0,2447	0,2508 n/c	
0,0061	0,2629	0,2447	0,2553 n/c	
0,0130	0,2551	0,2377	0,2449 n/c	
0,3003	0,3088	0,3173	0,2890	0,3091

<i>Ceratitis capitata</i>	<i>Rhagoletis cerasi</i>	<i>Drosophila borealis</i>	<i>Drosophila bifasciata</i>	<i>Drosophila incompta</i>
0,0046	0,0046	0,0046	0,0045	0,0044
0,0047	0,0047	0,0045	0,0047	0,0045
0,0046	0,0047	0,0047	0,0047	0,0047
0,0045	0,0046	0,0046	0,0045	0,0046
0,0043	0,0042	0,0043	0,0042	0,0042
0,0039	0,0040	0,0039	0,0040	0,0039
0,0040	0,0040	0,0039	0,0040	0,0039
0,0041	0,0041	0,0040	0,0042	0,0041
	0,0033	0,0036	0,0036	0,0036
0,1444		0,0037	0,0038	0,0036
0,1829	0,1965		0,0033	0,0031
0,1719	0,1911	0,1349		0,0032
0,1746	0,1896	0,1220	0,1274	
0,1733	0,1887	0,1360	0,1161	0,1275
0,1701	0,1839	0,1313	0,1130	0,1213
0,1501	0,1625	0,1278	0,1070	0,1138
0,1689	0,1833	0,1304	0,1083	0,1189
0,1684	0,1825	0,1306	0,1079	0,1181
0,1691	0,1835	0,1312	0,1086	0,1194
0,1704	0,1861	0,1346	0,1132	0,1239
0,1696	0,1854	0,1337	0,1090	0,1222
0,1717	0,1861	0,1315	0,1099	0,1228
0,1723	0,1871	0,1344	0,1122	0,1249
0,1683	0,1805	0,1332	0,1134	0,1219
0,1686	0,1812	0,1332	0,1130	0,1219
0,1650	0,1766	0,1332	0,1170	0,1209
0,1632	0,1744	0,1315	0,1133	0,1179
0,1630	0,1740	0,1310	0,1129	0,1179
0,1619	0,1729	0,1304	0,1125	0,1172
0,1581	0,1764	0,1277	0,1121	0,1193
0,1618	0,1774	0,1301	0,1177	0,1224
0,1612	0,1778	0,1298	0,1151	0,1214
0,1671	0,1764	0,1776	0,1689	0,1634
0,3412	0,3395	0,3489	0,3418	0,3403
0,3594	0,3642	0,3652	0,3604	0,3566
0,3382	0,3412	0,3438	0,3349	0,3326
0,3687	0,3718	0,3719	0,3655	0,3636
0,1205	0,1560	0,1307	0,1062	0,1103
0,1280	0,1589	0,1298	0,1003	0,1094
0,1672	0,1674	0,1479	0,1195	0,1289
0,1344	0,1596	0,1231	0,1109	0,1100
0,1368	0,1649	0,1230	0,1230	0,1081
0,1482	0,1699	0,1442	0,0889	0,1203
0,1528	0,1636	0,1325	0,1260	0,1221
0,1179	0,1075	0,1625	0,1532	0,1438
0,1497	0,1658	0,1380	0,1169	0,1247
0,2407	0,2397	0,2583	0,2308	0,2328
0,2521	0,2120	0,2464	0,2178	0,2350
0,2432	0,2629	0,2720	0,2690	0,2295
0,2432	0,2599	0,2720	0,2705	0,2310
0,2348	0,2551	0,2609	0,2623	0,2290
0,3399	0,3144	0,3371	0,3201	0,3314

<i>Drosophila triauraria</i>	<i>Drosophila suzukii</i>	<i>Drosophila baimaii</i>	<i>Drosophila santomea</i>	<i>Drosophila teissieri</i>
0,0045	0,0046	0,0061	0,0045	0,0045
0,0046	0,0046	0,0062	0,0046	0,0045
0,0046	0,0046	0,0062	0,0046	0,0046
0,0046	0,0046	0,0063	0,0046	0,0046
0,0042	0,0042	0,0055	0,0042	0,0042
0,0040	0,0039	0,0050	0,0040	0,0039
0,0039	0,0039	0,0053	0,0040	0,0040
0,0040	0,0040	0,0055	0,0040	0,0040
0,0035	0,0035	0,0048	0,0035	0,0035
0,0037	0,0035	0,0049	0,0037	0,0037
0,0032	0,0032	0,0044	0,0032	0,0031
0,0030	0,0030	0,0039	0,0029	0,0029
0,0032	0,0031	0,0041	0,0032	0,0031
	0,0027	0,0033	0,0027	0,0027
0,0942		0,0036	0,0025	0,0024
0,0734	0,0887		0,0037	0,0036
0,0976	0,0745	0,0890		0,0006
0,0981	0,0745	0,0887	0,0035	
0,0979	0,0750	0,0888	0,0017	0,0034
0,1001	0,0810	0,0923	0,0745	0,0748
0,1014	0,0789	0,0874	0,0732	0,0731
0,1010	0,0788	0,0902	0,0738	0,0739
0,1048	0,0783	0,0919	0,0743	0,0742
0,1003	0,0969	0,0914	0,0986	0,0986
0,0984	0,0961	0,0914	0,0981	0,0981
0,1169	0,1152	0,1051	0,1129	0,1127
0,1159	0,1122	0,1051	0,1095	0,1093
0,1157	0,1119	0,1048	0,1091	0,1089
0,1147	0,1111	0,1037	0,1081	0,1080
0,1134	0,1111	0,1032	0,1085	0,1081
0,1172	0,1137	0,1070	0,1119	0,1114
0,1155	0,1116	0,1063	0,1110	0,1102
0,1606	0,1569	0,1359	0,1569	0,1569
0,3392	0,3373	0,3098	0,3365	0,3363
0,3582	0,3579	0,3284	0,3545	0,3547
0,3327	0,3329	0,2973	0,3326	0,3327
0,3652	0,3630	0,3393	0,3602	0,3610
0,0831	0,0965	0,0688	0,0946	0,0946
0,0758	0,0912	0,0640	0,0917	0,0912
0,1093	0,0101	0,0983	0,0794	0,0785
0,1136	0,1145	0,1133	0,1082	0,1078
0,1181	0,1124	0,1043	0,1093	0,1099
0,1214	0,1135	0,1220	0,1174	0,1174
0,1210	0,1129	0,1094	0,1202	0,1210
0,1480	0,1466	0,1380	0,1425	0,1411
0,1341	0,1124	0,1213	0,1208	0,1213
0,2397	0,2318	0,2397	0,2200	0,2230
0,2321	0,2149	0,2120	0,2178	0,2206
0,2599	0,2584	0,2492	0,2325	0,2295
0,2614	0,2599	0,2508	0,2310	0,2280
0,2565	0,2536	0,2464	0,2290	0,2261
0,3258	0,2861	0,3144	0,3286	0,3258

<i>Drosophila yakuba</i>	<i>Drosophila melanogaster</i>	<i>Drosophila mauritiana</i>	<i>Drosophila simulans</i>
0,0045	0,0046	0,0046	0,0046
0,0045	0,0046	0,0047	0,0047
0,0046	0,0047	0,0047	0,0047
0,0046	0,0046	0,0046	0,0047
0,0042	0,0042	0,0041	0,0041
0,0040	0,0039	0,0039	0,0039
0,0040	0,0039	0,0039	0,0039
0,0040	0,0038	0,0039	0,0039
0,0035	0,0036	0,0034	0,0035
0,0037	0,0036	0,0036	0,0036
0,0032	0,0033	0,0031	0,0031
0,0029	0,0030	0,0029	0,0029
0,0031	0,0033	0,0031	0,0031
0,0027	0,0028	0,0028	0,0029
0,0025	0,0026	0,0025	0,0026
0,0036	0,0036	0,0036	0,0036
0,0004	0,0025	0,0025	0,0025
0,0006	0,0025	0,0024	0,0025
	0,0025	0,0025	0,0025
0,0750		0,0020	0,0021
0,0740	0,0471		0,0017
0,0746	0,0484	0,0330	
0,0751	0,0503	0,0360	0,0350
0,0990	0,1021	0,0995	0,0994
0,0986	0,1014	0,0990	0,0990
0,1135	0,1153	0,1126	0,1150
0,1099	0,1126	0,1097	0,1122
0,1096	0,1122	0,1092	0,1120
0,1086	0,1112	0,1084	0,1111
0,1087	0,1092	0,1063	0,1079
0,1121	0,1132	0,1109	0,1124
0,1112	0,1119	0,1098	0,1112
0,1573	0,1598	0,1580	0,1596
0,3366	0,3335	0,3367	0,3351
0,3550	0,3564	0,3567	0,3576
0,3330	0,3305	0,3309	0,3297
0,3606	0,3625	0,3620	0,3609
0,0956	0,0970	0,1016	0,1002
0,0926	0,0940	0,0980	0,0940
0,0802	0,0827	0,0827	0,0806
0,1078	0,1163	0,1159	0,1190
0,1099	0,1112	0,1187	0,1124
0,1180	0,1186	0,1174	0,1152
0,1210	0,1160	0,1179	0,1164
0,1435	0,1452	0,1487	0,1438
0,1219	0,1163	0,1174	0,1169
0,2200	0,2161	0,2240	0,2318
0,2178	0,1977	0,2120	0,2264
0,2325	0,2401	0,2416	0,2523
0,2310	0,2416	0,2447	0,2568
0,2290	0,2391	0,2406	0,2507
0,3286	0,3258	0,3371	0,3343

<i>Drosophila sechellia</i>	<i>Drosophila ananassae</i>	<i>Drosophila auraria</i>	<i>Drosophila lehmanae</i> *
0,0046	0,0046	0,0046	0,0044
0,0046	0,0047	0,0047	0,0046
0,0047	0,0046	0,0046	0,0046
0,0047	0,0047	0,0047	0,0045
0,0042	0,0043	0,0043	0,0041
0,0039	0,0038	0,0038	0,0038
0,0040	0,0039	0,0039	0,0039
0,0039	0,0040	0,0040	0,0038
0,0036	0,0035	0,0035	0,0035
0,0036	0,0037	0,0037	0,0037
0,0033	0,0032	0,0032	0,0033
0,0030	0,0030	0,0031	0,0031
0,0032	0,0033	0,0033	0,0031
0,0029	0,0029	0,0028	0,0031
0,0025	0,0028	0,0028	0,0029
0,0037	0,0037	0,0037	0,0042
0,0026	0,0029	0,0030	0,0030
0,0025	0,0029	0,0029	0,0029
0,0026	0,0029	0,0029	0,0030
0,0021	0,0029	0,0029	0,0030
0,0018	0,0028	0,0028	0,0029
0,0017	0,0029	0,0029	0,0030
	0,0029	0,0029	0,0031
0,1022		0,0004	0,0031
0,1018	0,0019		0,0031
0,1172	0,1113	0,1108	
0,1136	0,1076	0,1075	0,0240
0,1130	0,1072	0,1070	0,0235
0,1122	0,1067	0,1065	0,0227
0,1095	0,1072	0,1073	0,0716
0,1144	0,1111	0,1112	0,0751
0,1135	0,1103	0,1102	0,0729
0,1600	0,1565	0,1571	0,1537
0,3349	0,3381	0,3388	0,3288
0,3590	0,3566	0,3569	0,3456
0,3322	0,3302	0,3309	0,3222
0,3624	0,3654	0,3656	0,3566
0,1006	0,1011	0,1011	0,1187
0,0990	0,0976	0,0976	0,1094
0,0844	0,1114	0,1114	0,1338
0,1159	0,0974	0,0974	0,1005
0,1149	0,1118	0,1118	0,1049
0,1163	0,1197	0,1197	0,1140
0,1183	0,1183	0,1183	0,1144
0,1407	0,1449	0,1449	0,1373
0,1157	0,1224	0,1224	0,1185
0,2299	0,2367	0,2367	0,2338
0,2120	0,2034	0,2034	0,2350
0,2401	0,2584	0,2584	0,2462
0,2447	0,2629	0,2629	0,2447
0,2406	0,2522	0,2522	0,2377
0,3286	0,3229	0,3229	0,3201

<i>Drosophila sturtevantii central*</i>	<i>Drosophila sturtevantii south*</i>	<i>Drosophila sturtevantii north*</i>
0,0044	0,0044	0,0044
0,0046	0,0046	0,0046
0,0046	0,0046	0,0046
0,0045	0,0045	0,0045
0,0041	0,0041	0,0041
0,0038	0,0038	0,0038
0,0039	0,0039	0,0039
0,0039	0,0039	0,0039
0,0036	0,0036	0,0036
0,0037	0,0037	0,0037
0,0033	0,0033	0,0033
0,0031	0,0031	0,0031
0,0031	0,0031	0,0031
0,0031	0,0031	0,0031
0,0030	0,0030	0,0030
0,0042	0,0042	0,0042
0,0030	0,0030	0,0030
0,0029	0,0029	0,0029
0,0030	0,0029	0,0030
0,0030	0,0030	0,0030
0,0030	0,0029	0,0029
0,0030	0,0030	0,0030
0,0031	0,0031	0,0031
0,0031	0,0031	0,0031
0,0031	0,0031	0,0031
0,0015	0,0015	0,0014
	0,0000	0,0002
0,0000		0,0002
0,0006	0,0005	
0,0672	0,0665	0,0659
0,0719	0,0711	0,0706
0,0696	0,0690	0,0682
0,1495	0,1492	0,1488
0,3242	0,3239	0,3234
0,3442	0,3442	0,3432
0,3186	0,3181	0,3179
0,3569	0,3568	0,3563
0,1160	0,1156	0,1160
0,1073	0,1073	0,1077
0,1283	0,1277	0,1263
0,0907	0,0904	0,0900
0,1063	0,1058	0,1063
0,1079	0,1070	0,1084
0,1166	0,1159	0,1162
0,1378	0,1368	0,1371
0,1144	0,1149	0,1143
0,2330	0,2333	0,2330
0,2184	0,2206	0,2184
0,2432	0,2424	0,2416
0,2416	0,2409	0,2401
0,2362	0,2355	0,2348
0,3267	0,3258	0,3258

<i>Drosophila prosaltans</i> *	<i>Drosophila septentriosaltans</i> *	<i>Drosophila pseudosaltans</i> *	<i>Haematobia irritans</i>
0,0045	0,0045	0,0045	0,0045
0,0047	0,0048	0,0047	0,0046
0,0046	0,0046	0,0045	0,0045
0,0045	0,0045	0,0045	0,0046
0,0042	0,0041	0,0041	0,0041
0,0039	0,0038	0,0038	0,0038
0,0040	0,0040	0,0040	0,0038
0,0040	0,0040	0,0040	0,0038
0,0036	0,0036	0,0036	0,0035
0,0038	0,0038	0,0038	0,0036
0,0032	0,0032	0,0033	0,0036
0,0031	0,0031	0,0031	0,0037
0,0032	0,0032	0,0032	0,0036
0,0031	0,0031	0,0031	0,0035
0,0030	0,0030	0,0030	0,0034
0,0039	0,0040	0,0041	0,0045
0,0030	0,0030	0,0030	0,0035
0,0030	0,0030	0,0030	0,0035
0,0030	0,0030	0,0030	0,0035
0,0029	0,0029	0,0030	0,0035
0,0029	0,0030	0,0030	0,0035
0,0030	0,0031	0,0030	0,0035
0,0031	0,0031	0,0031	0,0036
0,0031	0,0031	0,0032	0,0036
0,0031	0,0031	0,0032	0,0036
0,0025	0,0025	0,0025	0,0036
0,0024	0,0024	0,0024	0,0036
0,0024	0,0024	0,0024	0,0036
0,0023	0,0024	0,0023	0,0036
	0,0014	0,0014	0,0035
0,0206		0,0015	0,0036
0,0228	0,0268		0,0035
0,1495	0,1540	0,1520	
0,3240	0,3262	0,3252	0,3320
0,3444	0,3461	0,3460	0,3487
0,3163	0,3180	0,3176	0,3253
0,3547	0,3576	0,3563	0,3571
0,1039	0,1076	0,1076	0,1140
0,1021	0,1053	0,1058	0,1194
0,1148	0,1215	0,1174	0,1577
0,0870	0,0888	0,0879	0,1222
0,1037	0,1093	0,1093	0,1255
0,1095	0,1129	0,1112	0,1266
0,1079	0,1141	0,1129	0,1363
0,1404	0,1411	0,1452	0,1304
0,1169	0,1169	0,1119	0,1386
0,2446	0,2446	0,2407	0,2132
0,2292	0,2264	0,2235	0,2206
0,2416	0,2447	0,2432	0,2204
0,2416	0,2447	0,2432	0,2249
0,2391	0,2391	0,2377	0,2232
0,3116	0,3116	0,3059	0,3031

<i>Nasonia vitripennis</i>	<i>Wiebesia pumilae</i>	<i>Kradibia gibbosae</i>	<i>Ceratosolen solmsi</i>	<i>Drosophila bicornuta</i>
0,0045	0,0046	0,0046	0,0051	0,0097
0,0048	0,0047	0,0048	0,0054	0,0093
0,0046	0,0047	0,0046	0,0052	0,0097
0,0046	0,0045	0,0043	0,0052	0,0096
0,0045	0,0046	0,0046	0,0050	0,0085
0,0047	0,0047	0,0046	0,0051	0,0082
0,0045	0,0046	0,0045	0,0050	0,0086
0,0049	0,0049	0,0050	0,0054	0,0096
0,0045	0,0044	0,0046	0,0049	0,0074
0,0045	0,0046	0,0047	0,0049	0,0079
0,0044	0,0044	0,0045	0,0049	0,0074
0,0045	0,0044	0,0047	0,0050	0,0068
0,0046	0,0044	0,0045	0,0051	0,0071
0,0046	0,0045	0,0046	0,0051	0,0061
0,0046	0,0045	0,0046	0,0050	0,0065
0,0061	0,0061	0,0061	0,0066	0,0056
0,0047	0,0045	0,0046	0,0051	0,0063
0,0047	0,0045	0,0046	0,0051	0,0062
0,0047	0,0045	0,0046	0,0050	0,0063
0,0046	0,0045	0,0046	0,0050	0,0064
0,0046	0,0045	0,0045	0,0049	0,0066
0,0046	0,0045	0,0046	0,0049	0,0065
0,0046	0,0046	0,0047	0,0050	0,0066
0,0047	0,0045	0,0047	0,0051	0,0066
0,0047	0,0045	0,0047	0,0051	0,0066
0,0046	0,0044	0,0046	0,0050	0,0070
0,0046	0,0044	0,0046	0,0050	0,0070
0,0046	0,0044	0,0046	0,0050	0,0070
0,0046	0,0045	0,0045	0,0051	0,0063
0,0046	0,0044	0,0046	0,0051	0,0065
0,0046	0,0044	0,0045	0,0050	0,0066
0,0045	0,0045	0,0044	0,0050	0,0068
	0,0044	0,0043	0,0050	0,0095
0,3082		0,0042	0,0049	0,0099
0,2774	0,2724		0,0048	0,0095
0,3221	0,3096	0,2812		0,0104
0,2677	0,2952	0,2624	0,3036	
0,2648	0,2958	0,2642	0,3080	0,0637
0,3061	0,3155	0,2924	0,3361	0,1058
0,2630	0,2995	0,2700	0,3108	0,1086
0,2715	0,3136	0,2844	0,3291	0,1137
0,3051	0,3464	0,2839	0,3532	0,1138
0,2945	0,3277	0,2893	0,3368	0,1153
0,2719	0,3000	0,2659	0,3215	0,1431
0,3001	0,3425	0,2951	0,3530	0,1198
0,1110	0,2233	0,1886	0,2465	0,2525
0,1203	0,2120	0,1948	0,2808	0,2493
0,2408	0,2715	0,2393	0,2899	0,2462
0,2393	0,2715	0,2408	0,2883	0,2477
0,2339	0,2646	0,2383	0,2836	0,2406
0,3059	0,3541	0,3286	0,3796	0,3286

<i>Drosophila nikananu</i>	<i>Drosophila subpulchrella</i>	<i>Drosophila tropicalis</i>	<i>Drosophila arawakana</i>
0,0097	0,0100	0,0096	0,0110
0,0093	0,0100	0,0096	0,0110
0,0094	0,0101	0,0096	0,0108
0,0092	0,0100	0,0095	0,0112
0,0087	0,0089	0,0088	0,0101
0,0083	0,0083	0,0083	0,0099
0,0085	0,0084	0,0083	0,0098
0,0098	0,0110	0,0100	0,0124
0,0075	0,0072	0,0071	0,0088
0,0080	0,0077	0,0074	0,0090
0,0074	0,0071	0,0069	0,0085
0,0065	0,0067	0,0067	0,0084
0,0068	0,0072	0,0063	0,0076
0,0058	0,0060	0,0068	0,0079
0,0063	0,0021	0,0066	0,0082
0,0053	0,0076	0,0073	0,0075
0,0062	0,0055	0,0067	0,0075
0,0062	0,0055	0,0066	0,0075
0,0062	0,0055	0,0066	0,0075
0,0060	0,0058	0,0066	0,0080
0,0064	0,0056	0,0067	0,0083
0,0062	0,0057	0,0069	0,0079
0,0064	0,0057	0,0069	0,0082
0,0064	0,0064	0,0063	0,0079
0,0064	0,0064	0,0063	0,0079
0,0069	0,0067	0,0062	0,0079
0,0069	0,0067	0,0060	0,0079
0,0069	0,0067	0,0060	0,0079
0,0069	0,0066	0,0060	0,0080
0,0065	0,0065	0,0058	0,0079
0,0066	0,0065	0,0060	0,0080
0,0067	0,0066	0,0058	0,0080
0,0070	0,0073	0,0070	0,0084
0,0096	0,0099	0,0094	0,0113
0,0100	0,0097	0,0097	0,0115
0,0095	0,0096	0,0094	0,0111
0,0100	0,0098	0,0098	0,0120
0,0052	0,0117	0,0082	0,0082
	0,0109	0,0080	0,0082
0,0945		0,0083	0,0122
0,0986	0,1185		0,0082
0,1104	0,1198	0,1118	
0,1114	0,1126	0,1129	0,1064
0,1124	0,1086	0,1068	0,0989
0,1453	0,1423	0,1593	0,1348
0,1173 n/c		0,1072	0,1078
0,2436	0,2453	0,2533	0,2402
0,2464 n/c		0,2286	0,2083
0,2568	0,2553	0,2492	0,2492
0,2568	0,2553	0,2492	0,2538
0,2522	0,2493	0,2406	0,2478
0,3201 n/c		0,3846	0,3768

<i>Drosophila neotestacea</i>	<i>Drosophila recens</i>	<i>Rhagoletis cingulata</i>	<i>Drosophila orientacea</i>	<i>Nasonia oneida</i>
0,0105	0,0086	0,0083	0,0108	0,0138
0,0107	0,0087	0,0081	0,0108	0,0136
0,0108	0,0086	0,0084	0,0107	0,0138
0,0110	0,0086	0,0084	0,0105	0,0131
0,0098	0,0079	0,0075	0,0097	0,0131
0,0094	0,0078	0,0072	0,0094	0,0133
0,0093	0,0077	0,0072	0,0094	0,0138
0,0114	0,0091	0,0083	0,0091	0,0222
0,0086	0,0070	0,0060	0,0086	0,0139
0,0088	0,0072	0,0057	0,0086	0,0136
0,0087	0,0066	0,0070	0,0083	0,0139
0,0074	0,0066	0,0070	0,0078	0,0141
0,0077	0,0064	0,0066	0,0078	0,0134
0,0078	0,0065	0,0068	0,0079	0,0137
0,0076	0,0063	0,0068	0,0072	0,0137
0,0079	0,0061	0,0067	0,0078	0,0140
0,0076	0,0063	0,0066	0,0075	0,0131
0,0076	0,0063	0,0066	0,0076	0,0132
0,0076	0,0063	0,0066	0,0075	0,0131
0,0079	0,0065	0,0068	0,0075	0,0131
0,0078	0,0065	0,0070	0,0074	0,0131
0,0079	0,0064	0,0069	0,0072	0,0135
0,0080	0,0065	0,0069	0,0073	0,0134
0,0080	0,0063	0,0064	0,0076	0,0134
0,0080	0,0063	0,0064	0,0076	0,0134
0,0076	0,0061	0,0066	0,0074	0,0135
0,0076	0,0063	0,0065	0,0075	0,0134
0,0076	0,0063	0,0064	0,0075	0,0134
0,0076	0,0063	0,0064	0,0074	0,0134
0,0077	0,0060	0,0064	0,0073	0,0137
0,0076	0,0061	0,0064	0,0074	0,0135
0,0076	0,0061	0,0065	0,0072	0,0136
0,0083	0,0068	0,0064	0,0084	0,0132
0,0109	0,0090	0,0082	0,0111	0,0098
0,0115	0,0093	0,0084	0,0112	0,0132
0,0109	0,0088	0,0084	0,0107	0,0124
0,0116	0,0095	0,0087	0,0111	0,0136
0,0089	0,0068	0,0077	0,0090	0,0141
0,0089	0,0068	0,0079	0,0089	0,0139
0,0126	0,0116	0,0092 n/c		0,0177
0,0088	0,0080	0,0084	0,0116	0,0160
0,0086	0,0080	0,0089	0,0119	0,0162
	0,0077	0,0103	0,0073	0,0186
0,1129		0,0082	0,0072	0,0139
0,1599	0,1586		0,0099	0,0132
0,0638	0,1113	0,1593		0,0239
0,2686	0,2515	0,2318	0,2270	
n/c	0,2292	0,2120	0,2250	0,1232
0,2695	0,2523	0,2538 n/c		0,2411
0,2711	0,2538	0,2523 n/c		0,2395
0,2755	0,2478	0,2478 n/c		0,2374
n/c	0,3286	0,3173	0,3119	0,3179

<i>Muscidifurax uniraptor</i>	<i>Nomada ferruginata</i>	<i>Nomada leucophthalma</i>	<i>Nomada panzeri</i>
0,0224	0,0180	0,0179	0,0174
0,0221	0,0185	0,0185	0,0180
0,0206	0,0174	0,0172	0,0167
0,0206	0,0031	0,0030	0,0043
0,0211	0,0181	0,0183	0,0176
0,0222	0,0173	0,0173	0,0166
0,0216	0,0172	0,0172	0,0167
0,0240 n/c	n/c	n/c	
0,0235	0,0176	0,0176	0,0169
0,0220	0,0176	0,0175	0,0170
0,0232	0,0183	0,0182	0,0173
0,0230	0,0183	0,0182	0,0173
0,0222	0,0173	0,0172	0,0167
0,0232	0,0186	0,0184	0,0178
0,0226	0,0179	0,0178	0,0172
0,0223	0,0180	0,0179	0,0173
0,0232	0,0173	0,0173	0,0167
0,0232	0,0173	0,0172	0,0167
0,0232	0,0173	0,0173	0,0167
0,0220	0,0171	0,0170	0,0165
0,0223	0,0174	0,0174	0,0168
0,0228	0,0180	0,0181	0,0173
0,0223	0,0172	0,0172	0,0166
0,0219	0,0173	0,0173	0,0167
0,0219	0,0173	0,0173	0,0167
0,0232	0,0174	0,0173	0,0168
0,0227	0,0176	0,0175	0,0168
0,0228	0,0176	0,0175	0,0168
0,0226	0,0176	0,0174	0,0168
0,0228	0,0176	0,0175	0,0169
0,0229	0,0176	0,0175	0,0168
0,0226	0,0178	0,0176	0,0170
0,0227	0,0169	0,0171	0,0165
0,0167	0,0165	0,0164	0,0160
0,0219	0,0174	0,0175	0,0172
0,0213	0,0171	0,0172	0,0168
0,0245	0,0180	0,0178	0,0173
0,0238	0,0176	0,0174	0,0166
0,0242	0,0175	0,0174	0,0168
n/c	0,0179	0,0177	0,0171
0,0421	0,0176	0,0175	0,0168
0,0437	0,0176	0,0177	0,0170
n/c	0,0185	0,0184	0,0183
0,0228	0,0179	0,0178	0,0172
0,0220	0,0178	0,0177	0,0171
0,0245 n/c	n/c	n/c	
0,0181	0,0175	0,0173	0,0171
n/c	n/c	n/c	
n/c		0,0030	0,0045
n/c	0,0061		0,0034
n/c	0,0137	0,0076	
0,3043 n/c	n/c	n/c	

Dactylopius coccus

	0,0237
	0,0248
	0,0246
	0,0241
	0,0246
	0,0253
	0,0245
	0,0253
	0,0253
	0,0244
	0,0252
	0,0249
	0,0251
	0,0246
	0,0237
	0,0247
	0,0249
	0,0249
	0,0249
	0,0248
	0,0250
	0,0250
	0,0247
	0,0248
	0,0248
	0,0244
	0,0246
	0,0245
	0,0245
	0,0240
	0,0240
	0,0242
	0,0249
	0,0237
	0,0256
	0,0258
	0,0267
	0,0251
	0,0250
n/c	
	0,0557
	0,0603
n/c	
	0,0246
	0,0246
	0,0260
	0,0248
	0,0257
n/c	
n/c	
n/c	