Supplementary material for Ko, W-Y, R. David, and H. Akashi, (2003) "Molecular phylogeny of the *Drosophila melanogaster* species subgroup", Journal of Molecular Evolution 57: 562-573

Supplementary material Table 1
Primers used for PCR Amplification and Sequencing

Gene	Name	Sequence (5'-3')	Note
Adh/Adhr	*Adh* 2235 +	GAAGGCAATCAATCCAAAGG	
	Adh 2274 -	GGGCTCAACATCCAACCA	
	Adh 2785 +	CTCATCCCACCCAGCCATCGTT	
	Adh 3248 -	CGCAATCCGCCACATAGCAGACAT	
	Adhr 3776 +	ATCCCCAGGCCATCGCTCAGT	
	Adh 2235	CCACCCTTATCCGCAATCCAT	
	Adh 2863 +	GAAACTGGACTTGGGCACCCTGGAG	
	Adh 2901 +	CCAAGCACTGGGACTCCGGCATCTAA	
	Adhr 3328 +	CATGACCAAGAACATAGCGGTGAGTG	
	Adhr 3253 -	CTCCGCAATCCGCCACATAGC	
	Adh 2313 -	TGACATCGTAGGGATAGAAGGTGAC	
	Adh 1932 +	GATCTTCCTATAAATACGGGGC	
	Adhr 4587 -	TTCACACYCCATAGATGACCCATA	
	Adhr 4481 -	ATTTAATCTTGGTCCTCCTCA	
	Adhr 3488 -	CCGAATCTAAGCCGAAACTGAAAC	
	Adhr 2260 -	AAGCTGAAGACCGTCGACATCCTG	for *D. lutescens*
	Adhr 3470 -	AATCTGAAACATAAACTTGGTAGTAG	for *D. lutescens*
	Adhr 2680 -	CAGCGTGGTGCGGGTGATGC	for *D. lutescens*
	Adhr 4290 +	GGCCTTTCTAGAATATGGACAATC	for *D. lutescens*
	Adhr 3981 +	CGATAAACCCGAGCACCCAGATA	for *D. lutescens*
Gld	*Gld* 414 +	GCCTCAGCCTGCGATTGTTTGG	
	Gld 649 -	CCGCCGCCAATGACAATGAAATC	
	Gld 1124 +	GCCCAGATACCCTCGATGTTCCT	
	Gld 1429 -	AGGYGGATTRTACGGGAACTTG	
	Gld 1440 -	ACAACCCACCGCTATCCTACGCCA	
	Gld 1455 +	TTCCCATAYAAYCCACCTCTATC	
	Gld 1474 -	GAGAAGCCCAACTCCTCGCCG	
	Gld 1623 -	GGTGGCCGTGGTGTTCAGCAG	
	Gld 1637 +	CTGCATATCCTGCTGAACACCAC	
	Gld 1692 -	CGCATGCTGCCGAAYTGGTCACTG	
	Gld 1972 +	TACTTGCTBTTCCGRGATGGTCTCA	
	Gld 1978 -	ACATCCGAAATGCCAGTGCC	
	Gld 2423 +	GCAGAACACGGGACCGGAGAATCA	
	Gld 2425 +	GCAGAAYACGGGTCCGGARAATCAC	
	Gld 2523 -	CCTGCGGCATTATACTGGTGT	for *D. eugracilis*
	Gld 2607 +	GAAGGGCGCCTACCTGCTGAAG	

	Gld 2635 -	CAACGTCCACGTCGCATCCAC	
ry	*ry* 2427 +	AGCTGCGSCTGTGCGGMACGA	
	ry 2506 -	TTCACCGCCAGGTGGCGTATCTTATTG	for *D. mimetica*
	ry 2805 +	CGAGGGMTACAAGACKTTTACCAAGGA	for *D. lutescens* and *D. eugracilis*
	ry 2831 +	TGCGGMATGGGCGAGAAG	
	ry 2877 -	GCTGCGCTCGAAGAGCTYGTCATC	
	ry 3123 -	GTGGGATTGATSAGGTGCGGGTAGAG	
	ry 3307 +	CCGTGGATATGCTTYACTACTTTGCCG	
	ry 3335 +	GGCAAGCAGATCCGCAAC	
	ry 3481 -	GATAACATTCCTGCGATAGCCRGTGAAGAA	
	ry 3781 +	TGGAGCGCGTGGCGGAGAG	
	ry 4067 -	GATAGCYTCACCGGTGGCCTGTTT	
	ry 4168 +	TTAGTACCAAGCCGCGTGCCAAG	for *D. eugracilis*
	ry 4336 +	GTGGGCGCCATAGCDGCCGATA	
	ry 4549 +	CGAATGGGCGGACAGGAGCACTTC	
	ry 4614 -	CTGAACCTCCGAGGGGTGTTGAGTGG	for *D. eugracilis*
	ry 4665 -	ACAGACGACACGRTGGGCAGGAAG	
	ry 4858 +	GGCATCCCTTCCTYTTCAA	
	ry 4908 -	CCGGCATTGTTGTAGCACTC	
	ry 4963 -	CAGATCCATGGACCAACCGGCATT	
	ry 5440 -	GTAGTGYGTATAGTCGCCWGTTTTGTAGAA	
	ry 5572 -	TTSCGCCAGCGATTCTCC	
	ry 5783 -	GTGGCCGTYTCCGAAATGTGAATCAG	
	ry 5955 +	GAGGCACCTGGRAGGAGTGGAT	
	ry 6004 +	CAGTCTCTCGGCCACYGGWTTCTA	
	ry 6038 -	TRTAGGTGCGRGCATTGGGATT	
	ry 6334 +	CCCGGGGAGTTYAATGTCAGC	
	ry 6447 -	AATGGCCTCCTTGATGG	for *D. lutescens* and *D. mimetica*
	ry 6553 -	CAATGTTCCACGGCGTAAATGAT	for *D. yakuba*

"+" or "-" in primer names indicate forward or reverse primer, respectively.

A

C20 5' CTCTCCCTTCTCGAATCGTAA 3'

B21 5' CGTAACCGTTCGTACGAGAAT 3'

D19 5' AATCGCTGTCCTCTCCTTC 3'

vect 57 3' GAGAGGGAAGAGAGCAGGCAAGGAATGGAAGCTGTCTGTCGCAGGAGAGGAAGXXXX 5'

B

For *Bam* HI, *Bcl* I, *Bgl* II

vect 53   5' CTCTCCCTTCTCGAATCGTAACCGTTCGTACGAGAATCGCTGTCCTCTCCTTC 3'
                ||||||||||| || || | | ||| | | |||||||||||||
vect 57 CTAG 3' GAGAGGGAAGAGAGCAGGCAAGGAATGGAAGCTGTCTGTCGCAGGAGAGGAAG CTAG 5'

For *Xba* I, *Nhe* I, and *Spe* I

vect 53   5' CTCTCCCTTCTCGAATCGTAACCGTTCGTACGAGAATCGCTGTCCTCTCCTTC 3'
                ||||||||||| || || | | ||| | | |||||||||||||
vect 57 GATC 3' GAGAGGGAAGAGAGCAGGCAAGGAATGGAAGCTGTCTGTCGCAGGAGAGGAAG GATC 5'

For *EcoR* I, *Apo* I, and *Mfe* I

vect 53   5' CTCTCCCTTCTCGAATCGTAACCGTTCGTACGAGAATCGCTGTCCTCTCCTTC 3'
                ||||||||||| || || | | ||| | | |||||||||||||
vect 57 TTAA 3' GAGAGGGAAGAGAGCAGGCAAGGAATGGAAGCTGTCTGTCGCAGGAGAGGAAG TTAA 5'

For *BsaH* I, *BstB* I, *Cla* I, *Taq*$^\alpha$ I, *Hpa* II, and *Nar* I

vect 53   5' CTCTCCCTTCTCGAATCGTAACCGTTCGTACGAGAATCGCTGTCCTCTCCTTC 3'
               ||||||||||| || || | | ||| | | |||||||||||||
vect 55 GC 3' GAGAGGGAAGAGAGCAGGCAAGGAATGGAAGCTGTCTGTCGCAGGAGAGGAAG GC 5'

Supplementary material Figure 1

Diagrams of vectorette DNA sequences and vectorette primers. Vectorette primers, C20, B21, and D19 were used for initial vectorette PCR, re-amplification, and sequencing, respectively (A). Double strand vectorettes used for ligation to digested genomic DNA (B). The vectorette sequences are modified from the Botstein laboratory website at Stanford University (http://genome-www.stanford.edu/group/botlab/index.html).

Supplementary material Figure 2

Gene specific phylogenetic trees for the *D. melanogaster* species group. Maximum–likelihood (ML), neighbor-joining distance (NJ), and maximum parsimony (MP) trees for *Adh*, *Adhr*, *Gld*, and *ry*. Abbreviations for species are: *D. melanogaster* (*mel*), *D. simulans* (*sim*), *D. teissieri* (*tei*), *D. yakuba* (*yak*), *D. erecta* (*ere*), *D. orena* (*ore*), *D. eugracilis* (*eug*), *D. mimetica* (*mim*), *D. lutescens* (*lut*), *D. pseudoobscura* (*pse*), and *D. subobscura* (*sub*). Percent bootstrap support (1000 replicates) is given on each node. Branch lengths are proportional to the scale, given in substitutions per nucleotide, for ML and NJ and numbers of substitutions for MP.