Available online at www.sciencedirect.com
SCIENCE DIRECT®
Molecular Phylogenetics and Evolution 30 (2004) 336–343
MOLECULAR PHYLOGENETICS AND EVOLUTION
www.elsevier.com/locate/ympev

Phylogenetic relationships of *Drosophila melanogaster* species group deduced from spacer regions of *histone* gene H2A-H2B

Yong Yang,[a] Ya-ping Zhang,[b,c] Yuan-huai Qian,[a] and Qing-tao Zeng[a,*]

[a] *College of Life Science, Hubei University, Wuhan 430062, China*
[b] *Laboratory of Cellular and Molecular Evolution, Kun-ming Institution of Zoology, the Chinese Academy of Science, Kunming 650223, China*
[c] *Laboratory for Conservation and Utilization of Bio-resource, Yunnan University, Kunming 650091, China*

Received 18 September 2002; revised 12 May 2003

Abstract

Nucleotide sequences of the spacer region of the *histone* gene H2A-H2B from 36 species of *Drosophila melanogaster* species group were determined. The phylogenetic trees were reconstructed with maximum parsimony, maximum likelihood, and Bayesian methods by using *Drosophila pseudoobscura* as the out group. Our results show that the *melanogaster* species group clustered in three main lineages: (1) *montium* subgroup; (2) *ananassae* subgroup; and (3) the seven oriental subgroups, among which the *montium* subgroup diverged first. In the third main lineage, *suzukii* and *takahashii* subgroups formed a clade, while *eugracilis*, *melanogaster*, *elegans*, *ficusphila*, and *rhopaloa* subgroups formed another clade. The bootstrap values at subgroup levels are high. The phylogenetic relationships of these species subgroups derived from our data are very different from those based on some other DNA data and morphology data.

Keywords: *melanogaster* species group; phylogenetic relationship; *histone* gene; H2A-H2B; Bayesian

1. Introduction

Drosophila melanogaster species group includes more than 160 species, most of which have been classified into 12 subgroups based on morphologic characters (Grimaldi, 1990; Lemeunier et al., 1986; Okada, 1954; Toda, 1991). Although some previous studies analyzed the phylogenies of *melanogaster* species group from morphology (Bock and Wheeler, 1972; Hsu, 1949), biogeography (Lemeunier et al., 1986; Throckmorton, 1975), chromosomal data (Ashburner et al., 1984), and molecular data (Goto et al., 2000; Harr et al., 2000; Inomata et al., 1997; Pelandakis and Solignac, 1993), the relationships among some subgroups, especially some species complex within *montium* subgroup, are still controversial. From the periphallic organs, Hsu (1949) considered that the *suzukii* subgroup was closest to the *Drosophila obscura* stem and the following two lines, *melanogaster–takahashii*, and *ananassae–montium*, his hypothesis was latterly supported by Okada (1954) who recognized three series from morphology, *suzukii*, *melanogaster–takahashii–ficusphila*, and *ananassae–montium*, however, was not entirely supported by Bock and Wheeler (1972). In addition, from the integration of chromosomal data, Ashburner et al. (1984) suggested that *melanogaster* species group had three lineages, *ananassae*, *montium*, and *melanogaster–takahashii–suzukii–eugracilis–ficusphiala–elegenas*, which well supported Bock's hypothesis. On the other hand, the most molecular evidence gave the similar results to Bock's result. The main hypotheses from DNA molecular are shown in Fig. 1. Pelandakis et al., 1991; Pelandakis and Solignac, 1993; Fig. 1A and analyzed eight subgroups based on the rDNA sequences data, and recognized five lineages, the *ananassae* subgroup was the ancestral subgroup followed by the *montium* subgroup, the

* Corresponding author.
E-mail addresses: yangyong@hubu.edu.cn (Y. Yang), zengqit@hubu.edu.cn (Q.-t. Zeng).

1055-7903/$ - see front matter © 2003 Elsevier Inc. All rights reserved.
doi:10.1016/S1055-7903(03)00212-4

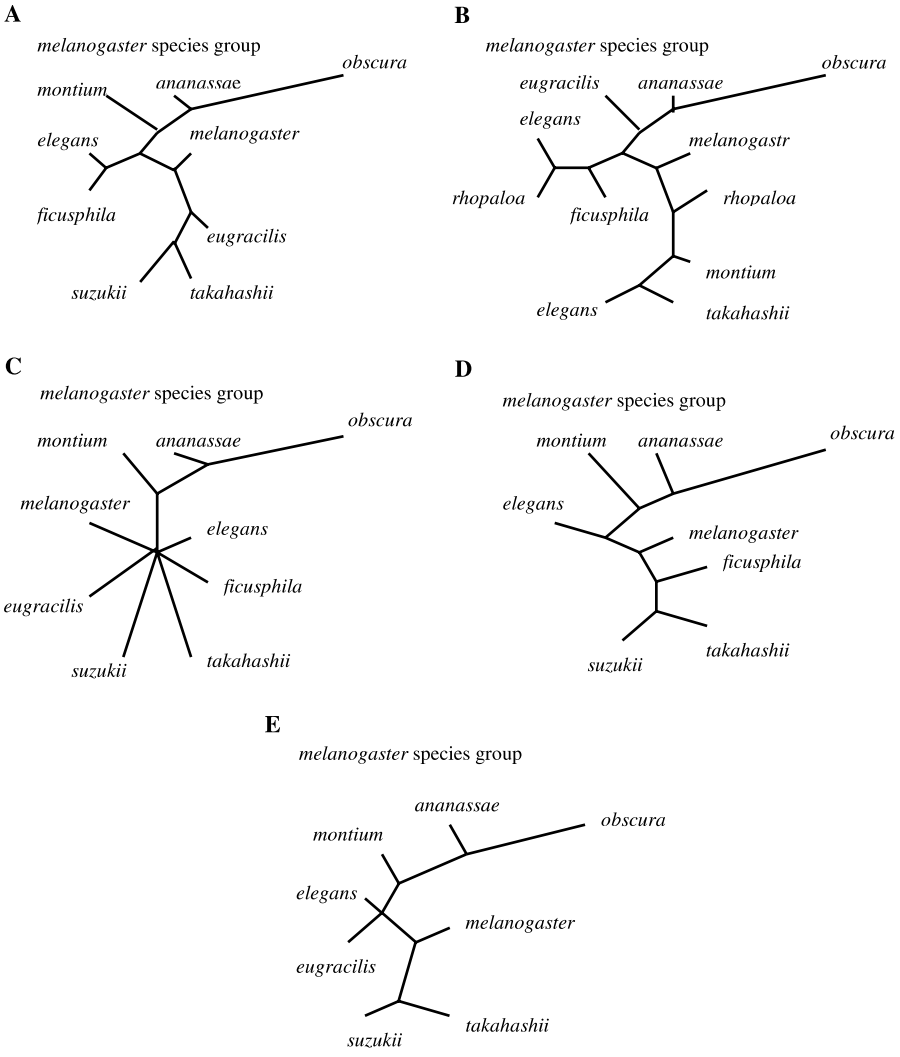

Fig. 1. The phylogenetic hypothesis inferred from rDNA (Pelandakis et al., 1991; Pelandakis and Solignac, 1993) (A); from *Amy* multigenes (Inomata et al., 1997) (B); from P transposable element (Clark et al., 1998) (C); from mitochondrial *COI* and nuclear *Gpdh* genes (Goto and Kimura, 2001) (D); and from microsatellite evolution data (Harr et al., 2000) (E).

ficusphila and *elegans* subgroups formed a clade as the sister group to the (*takkahashii–suzukii*)–*eugracilis* and *melanogaster* subgroups. Moreover, Inomata et al. (1997; Fig. 1B) inferred nine subgroups phylogenies from *Amy* multigenes, in contrast to Pelandakis and Solignac's hypothesis, they thought that *eugracilis* subgroup was close to *ananassae* subgroup, the other subgroups divided into two main lineages, however, the bootstrap values were very low in their trees. From P transposable element and comprehensive analysis data, Clark et al. (1998, Fig. 1C) suggested that *ananassae* is the first lineage, the *montiun* is the second lineage, and *melanogster*, *ficusphila*, *elegans*, *takahashii*, *suzukii*, and *eugracilis* divided at the same time after *montium* subgroup. The *COI* and *Gpdh* gene sequences (Goto and Kimura, 2001; Fig. 1D) revealed the phylogeny of seven subgroups, *ananassae*, *montium*, *melanogaster*, *elegans*, and *ficusphila*–(*suzukii–takahashii*). Harr et al. (2000) emphasized the phylogenetic relationships of the five so-called oriental subgroups: *melanogaster*, *elegans*, *eugracilis*, *suzukii*, and *takahashii* subgroups, and they suggested that the *melanogaster*, *takahashii*, and *suzukii* subgroups formed a monophyletic clade, *elegans* and *eugracilis* were polyphyletic clade (shown in Fig. 1E). From the previous studies, we can see that the numbers of the subgroups are limited and the relationships of the so-called oriental subgroups are still controversial. It is necessary to get new genetic data and more subgroup species to uncover these questions.

The spacer region of the divergently transcribed *histone* gene pair H2A and H2B was a good genetic maker for *Drosophila* phylogenetic relationships analysis (Baldo et al., 1999). We have therefore decided to use these regions to analyze the phylogenic relationships among 36 species from nine subgroups of *Drosophila melanogaster* species group.

2. Materials and methods

2.1. Species chosen

Most species in this study were collected in China and some kindly provided by Professors Watabe and Toda. Information about the name, locality, and GenBank accession numbers of the specimens are shown in Table 1.

2.2. DNA extraction, amplification, and sequencing

Total DNA was extracted from one fresh adult fly and stored at −20 °C for latter PCR amplifications. The primers for amplification and sequencing of the spacer regions of H2A and H2B are from Baldo et al. (1999). Primers locate in highly conserved regions of the H2A and H2B genes (all primers in 5′–3′ direction): H2AF (GCAGCATTGCCAGCCAACT) and H2BR (CTGT

Table 1
Experimental species, complex, subgroup, and distribution for H2A-H2B analysis

Subgroup	Complex	Species	Location	Accession Nos.
montioum	*auraria*	*D. auraria*	Riyuan, Japan	AY147418
		D. biauraria	Jilin, China	AY147420
		D. quadraria	Taiwan, China	AY147421
		D. triauraria	Shanghai, China	AY147424
		D. tani	Wuhan, China	AY147423
		D. rufa	Japan	AY147422
		D. punjabiensis	Mandalay, Burma	AY147438
		D. seguyi	Nairobi, Kenya	AY147429
	kikkawai	*D. bocki*	Wau(PNG), Japan	AY147427
		D. kikkawai	Wuhan, China	AY147435
		D. leontia	Nagarahole, India	AY147436
		D. barbarae	Maynyo (Burma)	AY147428
		D. birchii	Wuhan, China	AY147425
		D. jambulina	Nagarahole, India	AY147434
		D. serrata	Noumea (New Ledonia)	AY278407
	sp	*D. baimaii*	Hainan, China	AY147426
		D. madikerii	Japan	AY278408
melanogaster	*melanogaster*	*D. yakuba**		AJ224809
		*D. mauritiana**		AJ224806
		*D. simulans**		AJ224807
		*D. melanogaster**		AJ224808
takahashii	*takahashii*	*D. liui*	Yunnan, China	AY147447
		D. takahashii	Hunan, China	AY147449
		D. prostipennis	Guangdong, China	AY147448
		D. trilutea	Guangdong, China	AY147440
suzukii		*D. pulchrella*	Hunan,China	AY147446
		D. suzukii	Wuhan, China	AY147445
		D. biarmipes	Hainan, China	AY147433
ficusphila		*D. ficusphila*	Guangdong, China	AY147443
rhopaloa		*D. fuyamai*	Japan	AY147444
		D. prolongata	Yunnan, China	AY147439
elegans		*D. elegans*	Haina, China	AY147441
eugracilis		*D. eugracilis*	Yunnan, China	AY147442
ananassae	*ananassae*	*D. ananassae*	Inner Mongolia, China	AY147431
	bipectiuata	*D. malerkotliana*	Hubei, China	AY278409
		D. parabipectinata	Hannan, China	AY147430
		D. bipectinata	Wuhan, China	AY147432
obscura	*obscura*	*D. pseudoobscura**		AJ224812

The sequences of the species marked with * were from Baldo et al. (1999).

TCATTATGCTCATCGCCTT). PCRs were performed at the following conditions: the total volumes are 50 μl containing 1.5 U AmpliTaq DNA polymerase, 5 μl repliterm buffer (10×), 1.5 mM $MgCl_2$, 2 μl dNTP (1 nM), 1 μl primers (10 pM), 30 ng template DNA. Amplification was implement with denaturing at 95 °C for 3 min, 30 cycles of denaturing at 94 °C for 40 s, annealing at 50 °C for 40 s, and extension at 72 °C for 1 min and 20 s, followed by extension at 72 °C for 5 min. The amplification fragments were purified with the Qiaquick PCR Purification Kit (America FMC). Each sample was sequenced at least two times to assure accuracy. Sequencing reaction was carried out on a thermal cycler (Amplitron, I, Barnstead or Thermolyne) with the Bigdye sequencing Kit (ABI, No. 402079 Perkin–Elmer), the reaction and programs were implemented according to the recommendation of the handbook in the same thermal cycles, then electrophoresis in a ABI377 sequencer (Perkin–Elmer).

2.3. Sequence alignment and phylogenetic analysis

Sequences were aligned by CLUSTAL W program (Thompson et al., 1994), multiple alignment parameters were selected as the following: gap open penalty 15, gap extension penalty 6.66, transition weight 0.5, delay divergent sequence 30%, and finally the alignment was manually adjusted. The base frequencies, composition, and divergence values were calculated with MEGA 2.0 (Kumar et al., 2001). Nonstationary nucleotide composition was tested for the total base composition by the X^2 homogeneity test in PAUP* (Swofford, 1998).

In parsimony analysis, the single gaps and gaps that aligned unambiguously forming larger indels were scored as "missing data," the characters state were treated as unorder. MP trees were constructed in PAUP* (Swofford, 1998) by running the heuristic search with TBR branch swapping, 100 random addition sequence replications, and non-parameter bootstrap resampling procedures were applied to get the coincidence of MP trees.

Bayesian analysis were performed in MrBayes 2.01 (Huelsenbeck and Ronquist, 2001) with general-time-reversible + gamma + invariants (GTR + G + I) model of sequence evolution and four Markov chain Monte Carlo (MCMC) sampling to assess phylogenetic relationships. We set the parameters in MrBayes as following: nst = 6, rate = gamma, basefreq = estimate, generations = 2,000, 000, and the posterion probability and branches of the phylogeny are summed by burnin = 500 and contype = allcompat.

In maximum-likelihood (ML) analysis, we removed the ambiguous from ML analysis, ModelTest 3.06 program (Posada and Crandall, 1998) was used to determine an appropriate ML model, then chose the most fit model to reconstruct the ML tree in PAUP* (Swofford, 1998). The ModelTest 3.06 program gave two different results under two different criteria, hierarchical likelihood ratio test (hLRTs) and Akaike information criterion (AIC) (see Table 2), we selected the parameter assumptions of the hLRTs criterion to construct the maximum likelihood tree, the process of tree construction and bootstrap replicates were implemented under the same conditions as described in MP analysis.

We considered the published results from the 28s rRNA regions (541 bp; divergent domain D2) (Pelandakis et al., 1991 and Pelandakis and Solignac (1993)), *Gpdh* and *COI* (Goto and Kimura, 2001) as well as combined analysis of nuclear and mitochondrial sequences (O'Grady and Kidwell, 2002) which all considered the *obscura* group as the sister group to *melanogaster* species group, so we selected one member of the *obscura* species group, *D. pseudoobscura*, as the out group in all analyses.

3. Results

3.1. Composition of the H2A-H2B spacer regions

The spacer regions of the divergently transcribed gene pair H2A and H2B of different subgroups in

Table 2
The results of model testing of evolution

	Hierarchical likelihood ratios (hLRTs)			Akaike information criterion (AIC)		
Model selected	GTR + I + G			K81uf + I + G		
$-\ln L$	14073.5615			8892.8486		
Base frequency	A = 0.4137; C = 0.1084; G = 0.1113; T = 0.3400			A = 0.4392 ; C = 0.1055; G = 0.1120; T = 0.3433		
Substitution model	$a = 0.7835$; $d = 0.9514$;	$b = 4.0791$; $e = 4.0791$;	$c = 1.0916$; $f = 1.000$	$a = 0.1000$; $d = 1.2476$;	$b = 4.7159$; $e = 4.7159$;	$c = 1.2476$; $f = 1.000$
Among site rate variation	I = 0.2491 G = 0.7509			I = 0.2491 G = 0.7509		
Gamma distribution shape parameter	$\alpha = 1.8901$			$\alpha = 1.8924$		

melanogaster species group were analyzed. These spacers show a length variation between 479 and 518 bp, the overall frequency of A, T, C, and G are 29.8, 23.1, 23.0, and 24.1, respectively. Distance values are generally lower in the same species subgroup than different subgroups (data not shown). These regions have no significant variations in nucleotide composition across taxa ($P = 1.000$).

3.2. Phylogenetic relationships among subgroups

Fig. 2 shows the strict consensus MP tree and the completely identical nodes in MP, Bayesian, and ML analysis. In the tree, the *melanogaster* species group clustered into three main lineages, and the *montium* subgroup is the first lineage, the *ananassae* subgroup is the second lineage, the oriental subgroups: *melanogaster*, *rhopaloa*, *suzukii*, *elegans*, *eugracilis*, *takahashii*, and *ficusphila* formed the third lineage. All of the subgroups are apparent monophyletic with high bootstrap values. In the oriental subgroups lineage, *suzukii* and *takahashii* subgroups formed a clade, while the *eugracilis*, *melanogaster*, *elegans*, *ficusphila*, and *rhopaloa* subgroups formed another clade. In the second clade, the *eugracilis* diverged first, followed by separation between *melanogaster* and *rhopaloa* + (*elegans* + *ficusphila*).

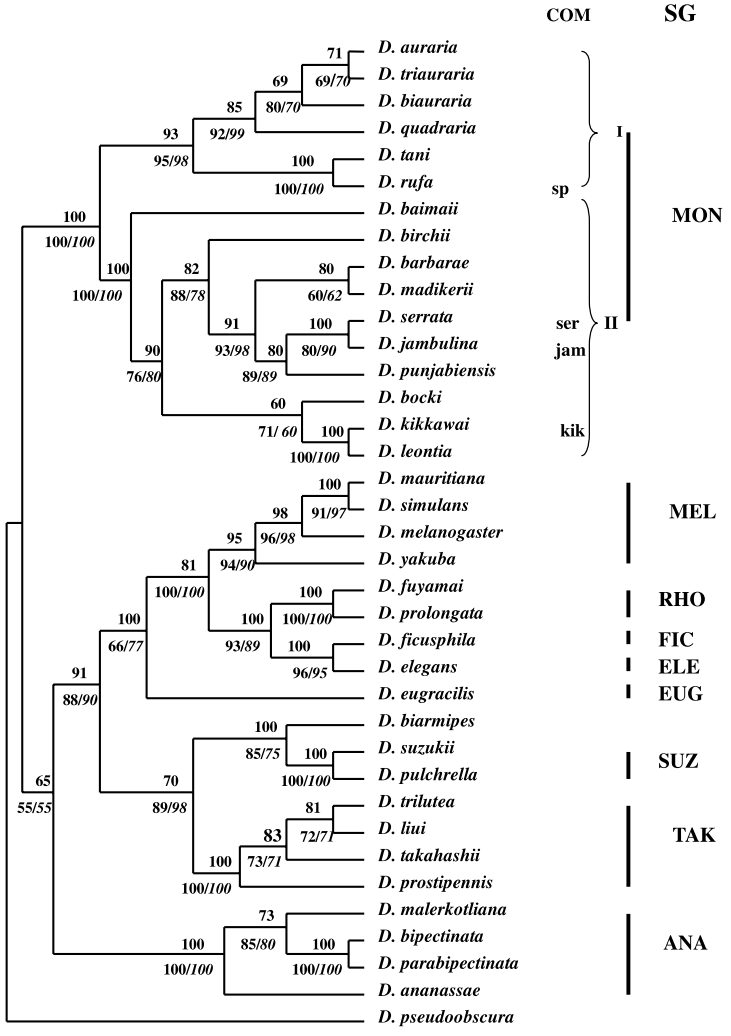

Fig. 2. The phylogenetic relationships of *melanogaster* species group based on spacers of *histone* gene H2A-H2B sequences by using maximum parsimony, Bayesin, and maximum-likelihood analysis. Numbers on the branches are the confidence scores evaluated by the bootstrap procedure with 1000 replicates in MP analysis; numbers under the branches are posterion probabilities in Bayesian analysis with MCMC algorithm; the italic numbers are bootstrap values in ML analysis. Taxa are indicated in the right: taxa having the rank of subgroup (SG): *ananassae* (ANA), *montium* (MON), and seven oriental subgroups: *melanogaster* (MEL), *eugracilis* (EUR), *suzukiii* (SUZ), *takahashii* (TAK), *elegans* (ELE), *ficusphila* (FIC), *rhopaloa* (RHO); taxa having the rank of complex (COM): *kikkawai* (kik), *auraria* (aur), *jambulina* (jam), *serrata* (ser), *seguyi* (seg), and unclassified species (sp). Tree length, the consistency index, and retention index in MP analysis are 2613, 0.6002, and 0.7819, respectively.

3.3. Phylogenetic relationships within montium subgroup

All of the three analysis methods gave similar phylogenetic relationships within each subgroup, and most supporting values are higher than 60%. The *montium* subgroup apparently cluster into two clades (here we gave the I and II symbols, respectively, in Fig. 2), (I) is composed of *D. rufa*, *D. tani*, and the *auraria* species complex, in the *auraria* complex, the *D. quadraria* branched first off from *auraria* complex followed by the *D. biauraria*, then the *D. auraria* and the *D. triauraria*. Another (II) is composed of the remaining species. One member of the unclassified species, *D. baimaii*, was moredivergent and separated earlier than the others, followed by the *kikkawai* complex species, then the *D. birchii*, *D. madikerii*, *D. punjabienis*, *D. jambulina*, and *D. serrata*.

4. Discussion

In our experiments, the different strain of one species analyzed gave the same sequence and we have sequenced several independent copies of the H2A-H2B regions for each species, low polymorphism within species was found.

4.1. Phylogenetic relationships among subgroups

Analysis of the H2A-H2B regions from 36 species in *melanogaster* species group and one species in *obscura* group reveals that the nine subgroups in the *melanogaster* species group rather closely related. The *montium* subgroup firstly branched off followed by the *ananassae* subgroup, the oriental subgroups branched off next. The branching pattern of the three lineages is not good agreement with the morphological and chromosomal data (Ashburner et al., 1984; Lemeunier et al., 1986), and the DNA data (Clark et al., 1998; Goto and Kimura, 2001; Inomata et al., 1997; Lage et al., 1996; Pelandakis and Solignac, 1993).

The *montium* subgroup is the biggest subgroup in the *melanogaster* species group and most members are cosmopolitan in distribution, the large numbers and comprehensive distribution make it become possible that the subgroup deduce more and more new species, so the *montium* subgroup may be the common ancestor of the *melanogaster* species group, and in our analysis, *montium* subgroup is close related to the *obscura* group, the *ananassae* as the second divergent lineage, and the supporting values are all high. The summary of P element evolution and chromosomal data plus molecular data shows that the *montium* subgroup separated earlier than the *ananassae* subgroup (Clark et al., 1998).

The relationships of *takahashii* and *suzukii* subgroups are well supported as a monophyletical clade in MP, Bayesian, and ML analysis, the supporting values are all higher than 70%, in additional, our results completely match the previous hypotheses deduced from morphological and chromosomal data (Ashburner et al., 1984; O'Grady and Kidwell, 2002), microsatellite evolution data (Harr et al., 2000) and the DNA sequences data (Clark et al., 1998; Goto and Kimura, 2001; Inomata et al., 1997; Lage et al., 1996; Pelandakis and Solignac, 1993). Inomata et al. (1997) suggested that the *takahashii* subgroup was close related to *elegans* subgroup, there was one limitation in their analysis, *suzukii* subgroup was ignored.

The *eugracilis* subgroup is unstable in previous reports. Pelandakis et al. (1991), Pelandakis and Solignac (1993) suggested that *eugracilis* sister to *melanogaster*, however, Inomata et al. (1997) assumed that *eugracilis* separated after *ananassae*, but the confidence value was very low (BP = 41%). *eugracilis* is clearly polyphyletic in Harr's analysis (2000) which maybe due to the outgroup selection. In our analysis, *eugracilis* was well supported as the sister group to *melanogaster* (BP = 63%, 100%, PP = 100%).

Goto et al. (2000) concluded that *melanogaster* subgroup branched off after the *montiun* subgroup, however, it is not the case, the position of *melanogaster* subgroup was variable in their different analysis methods and the *eugracilis* was absent in their analysis, the unstable topologies were because of smaller sample selection. Clark et al. (1998) suggested that the *melanogaster* subgroup and the five oriental subgroups cluster together with parallel relation, In their results, P element sequences are very different between *ananassae* subgroup, *montium* subgroup and the other subgroups in which are similar sequences. That the parallel relation is whether come from the horizontal transfer of P element between these subgroups or not is not clear. In our analysis, *melanogaster* subgroup is well supported as the sister group to *rhopaloa*, *ficusphila*, and *elegans* subgroups.

The *rhopaloa* subgroup clustered together with *elegans* and *ficusiphila* subgroups with high supporting values (BP = 82, PP = 85), however, this subgroup always was ignored in the previous studies except in Inomata's analysis with very low bootstrap value (Inomata et al., 1997). In their NJ tree, *rhopaloa* is closed to *ficusphila* and *elegans*. The phylogenetic position of *rhopaloa* subgroup is completely identical in MP, ML, and Bayesian analysis, based on the correct model of sequences evolution, likelihood methods are statistically consistent and powerful tools for resolving complex phylogenetic problems (Whelan et al., 2001), the result in Bayesian analysis strengthens the confidence in ML analysis, so we think that *rhopaloa* is closely related to *elegans* and *ficusphila* and the results is good consistent with Inomata's result (Inomata et al., 1997).

We think that the *ficusphila* and *elegans* subgroups have close relations (BP = 100%, 100%, PP = 100%) which was consistent to the results based on the D2 of 28S rRNA data (Pelandakis et al., 1991, Pelandakis and Solignac, 1993), however, the resemblance of the *elegans* and the *suzukii* did not make them cluster together (Bock, 1980), in additional, it is reported that the *ficusphlia* is close to the *melanogaster*, *suzukii*, and *takahashii* subgroups (Ashburner et al., 1984; Inomata et al., 1997), but Ashburner's results lacked the detailed analysis process, and only analyzed the chromosomal data of the *melanogaster* species subgroup, then give the final conclusions from comprehensive analysis based on part chromosomal data and the previous data, the *Amy* multigenes analysis as the coding duplicate gene copies in phylogenetic analyses is problematic when some taxa included in the data matrix contain duplicated paralogous sequences and other taxa contain ancestral unduplicated sequences. Lage et al. (1996) suggested that the Amylase genes undergo similar selective pressure and the sequences come being to homogenous. E.g. the sequence of *D. takahashii* was similar to that of *D. microlabis* in *obscura* species group, and in their MP tree *D. takahashii* cluster with *D. microlabis*.

4.2. The phylogenies within montium subgroups

The *montium* subgroup is the biggest subgroup (about 80 species) among oriental of Afritropical regions, but the center of the primary radiation seems to have been in the southeast Asia (Bock and Wheeler, 1972). The distribution and so many species make it very difficult to analyze the phylogeny of this subgroup. In the present experiment, 16 species were divided unambiguously into two main lineages (for convenient discussion, we gave the symbols I and II in all trees) *D. rufa*, *D. tani*, all species of *auraria* complex were clustered in one clade (I), species of *kikkawai* complex, *serrata* complex, and *jambulina* complex and some unclassified species were clustered in another clade (II). This result was not compatible with the prediction done by biochemical data (Ohnishi and Watanabe, 1984), partially DNA data (Goto et al., 2000; Nikolaidis and Scouras, 1996), and reproductive isolations data (Kim et al., 1989). In the I clade, the two unclassified species, *D. tani* and *D. rufa*, and the *auraria* species complex formed a monophyletical clade with high confidence values (PP = 100%, BP = 100%), which are high agree with the previous studies (Goto et al., 2000; Kim et al., 1993; Nikolaidis and Scouras, 1996; Ohnishi and Watanabe, 1984). In addition, *D. tani* and *D. rufa* are very similar to the species of *auraria* complex, the two species should be the members of *auraria* species complex. The *auraria* complex is very curious and the previous analysis always yield conflicting results (Dai and Liu, 1994; Kimura, 1987; Kurokawa, 1967; Lee, 1974). We got consistent results from the previous studies (Dai and Liu, 1994; Kim et al., 1989; Lee, 1974; Ohnishi and Watanabe, 1984). The *D. quadraria* is the ancestor species of the *auraria* complex, all of the other members deduced from *D. quadraria* (BP = 87%, PP = 88%).

On the other hand, in the II clade, *D. baimaii* was first branched off, the *D. kikkawai*, *D. leontia*, *D. bockii*, and some other species were very similar in morphology and have been termed "*kikkawai* complex" (Bock, 1980) and the four species in our study clustered together and confirmed an dependent clade. It is strange that the *D. barbarae*, one member of *kikkawai* complex from morphologic characters, do not cluster with the other members of *kikkawai*, but confirmed one independent clade with *D. madikerii*. The *jambulina* complex including *D. punjabienis* and *D. jambulina*, and *serrata* complex have closely relations as *Gpdh* and *COI* information shown (Goto and Kimura, 2001).

Acknowledgments

We are very grateful to Prof. Toda, M.J. and Prof. Wataba, H.A. for species diagnosis and Prof. F.-Y, Ling and Ms. B.H, Jin, Shou-tao, Li for maintaining fly stocks. We thank Dr. J.G., X.-Yu, J.F., Pang, and Y.G., Yao for critically reading the manuscript. This work was supported by the Natural Sciences Foundation of China (No. 39930100), and the Chinese Academy of Sciences.

References

Ashburner, M., Bodmer, M., Lemeunier, F., 1984. On the evolutionary relationships of *Drosophila melanogaster*. Dev. Genet. 4, 295–312.

Baldo, A.M., Les, D.H., Strausbaugh, L.D., 1999. Potentials and limitations of *histone* repeat sequences for phylogenetic reconstruction of *Sophophora*. Mol. Biol. Evol. 16 (11), 1511–1520.

Bock, I.R., Wheeler, 1972. The *Drosophila melanogaster* species group studies in genetics VII. Univ. Texas Publ. 7213.

Bock, I.R., 1980. Current status of the *Drosophila melanogaster* species group (Diptera). Syst. Entmol. 5, 341–356.

Clark, J.B., Kim, P.C., Kidwell, M.G., 1998. Molecular evolution of *P* transposable elements in the genus *Drosophila*. III. The *melanogaster* group. Mol. Biol. Evol. 15 (6), 746–755.

Dai, Z.H., Liu, F.L., 1994. Study on evolutionary genetics of *Drosophila auraria* species complex-cladistic analysis and phenetic analysis. Acta Genet. Sin. 21 (5), 362–372.

Goto, S.G., Kitamura, H.W., Kimura, M.T., 2000. Phylogenetic relationships and climatic adaptation in the *Drosophila takahashii* and *montium* species subgroups. Mol. Phylogenet. Evol. 15 (1), 147–156.

Goto, S.G., Kimura, M., 2001. Phylogenetic utility of mitochondrial *COI* and *COII* nuclear *Gpdh* genes in *Drosophila*. Mol. Phylogenet. Evol 18 (3), 404–422.

Grimaldi, T., 1990. A phylogenetic revised classification of genera in the *Drosophildae* (Diptera). Bull. Am. Mus. Nat. Hist. 197, 1–139.

Harr, B., Zangerl, B., Schlotterer, C., 2000. Removal of Microsatellite interruptions by DNA replication slippage: phylogenetic evidence from *Drosophila*. Mol. Biol. Evol. 17 (7), 1001–1009.

Hsu, T.C., 1949. The external genital apparatus of male *Drosophila* in relation to systematics. Univ. Texas Publ. 4920, 80–142.

Huelsenbeck, J.P., Ronquist, F., 2001. MRBAYES: Bayesian inference of phylogenetic trees. Bioinformatics 17 (8), 754–755.

Inomata, N., Tachida, H., Yamazaki, T., 1997. Molecular evolution of the *Amy* multigenes in the subgenus of male *Drosophila*. Mol. Biol. Evol. 14, 942–950.

Kim, B.K., Watanabe, T.K., Kitagawa, O., 1989. Evolutionary genetics of the *Drosophila montium* subgroup 1. Reproductive isolations and phylogeny. Jpn. J. Genet. 64, 177–190.

Kim, B.K., Aotsuka, T., Kitagawa, O., 1993. Evolutionary genetics of the *Drosophila montium* subgroup. II. mitochonary DNA variation. Zool. Sci. 10, 891–996.

Kimura, M.T., 1987. Hbitat differentiation and speciation in the *Drosophila auraria* species complex (Diptera, Drosophilidae). Kontyu 55, 429–436.

Kumar, S., Tamura, K., Jakobsen, I.B., Nei, M., 2001. MEGA2: Molecular Evolutionary Genetics Analysis software. Arizona State University, Tempe, AZ.

Kurokawa, H., 1967. Population genetics on three races of *Drosphila auraria* Peng III. Geographical and ecological distribution of the races A, B and C with species regard to its speciation. Jpn. J. Genet. 42, 109–119.

Lage, J.-L.D., Wegnez, M., Cariou, M.-L., 1996. Distribution and evolution of introns in *Drosophila* amylase genes. J. Mol. Evol. 43, 334–347.

Lee, T.J., 1974. Speciation oin the species complex *Drosophila auraria*. Jpn. J. Genet. 49, 305 (abstract).

Lemeunier, F., David, J.R., Tsacas, L., 1986. The *melanogaster* species group. In: Ashburner, M., Carson, H.L., Thompson, J.N. (Eds.), Genetics and Biology of *Drosophila*, 3e. Academic Press, London, pp. 148–256.

Nikolaidis, N., Scouras, Z.G., 1996. The *Drosophila montium* subgroup species. Phylogenetic relationships based on mitochondrial DNA analysis. Genome 39, 874–883.

Okada, T., 1954. Comparative morphology of *Drosophilid* flies. I. Phallic organs of the *melanogaster* group. Kontyuû 22, 36–46.

Ohnishi, S., Watanabe, T.K., 1984. Systemmatics of the *Drosophila montium* species subgroup: a biochemical approach. Zool. Sci. 1, 801–807.

O'Grady, P.M., Kidwell, M.G., 2002. Phylogeny of the subgenus *sophophora* (Diptera: Drosophilidae) based on combined analysis of nuclear and mitochondrial sequences. Mol. Phylogenet. Evol. 22, 442–453.

Pelandakis, M., Higgins, D.G., Solignac, M., 1991. Molecular phylogeny of the subgenus *Sophophora* of *Drosophila* derived from large subunit of ribosomal RNA sequences. Genetica 84, 87–94.

Pelandakis, M., Solignac, M., 1993. Molecular phylogeny of *Drosophila* based on ribosomal RNA sequences. J. Mol. Evol. 37, 525–543.

Posada, D., Crandall, K., 1998. MODELTEST: testing the model of DNA substitution. Bioinformatics 14 (9), 817–818.

Swofford, D.L., 1998. PAUP*. phylogenetic analysis using parsimony (* and other methods) Version 4. Associates, Sinauer, Sunderland, MA.

Thompson, J.D., Higgins, D.G., Gibson, T.J., 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequences weighting, position specific gap penalties and weight matrix choice. Nucleic Acids Res. 22, 4673–4680.

Throckmorton, L.H., 1975. The phylogeny, ecology, and geography of *Drosophila*. In: King, R.C. (Ed.), Handbook of Genetics, 3. Plenum, New York, pp. 421–469.

Toda, M.J., 1991. *Drosophila* (Diptera) in Myanmar (Burma) VII. The *Drosophila melanogaster* species group, excepting the *montium* species subgroup. Oriental Insects 25, 69–94.

Whelan, S., Lio, P., Goldman, N., 2001. Molecular phylogenetics: state-of-the-art methods for looking into the past. Trends Genet. 17 (5), 262–272.