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Drosophila hubeiensis (Diptera, Drosophilidae), a New Species of the *Drosophila obscura* Species-group from the Mainland of China

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Abstract *Drosophila* (*Sophophora*) *hubeiensis*, a new species of the *D. obscura* species-group from Hubei Province in Central China is described, and compared to its sibling species *D. sinobscura* inhabiting Taiwan island. The larval ganglion cells of *D. hubeiensis* possess a diploid number of 10 chromosomes (3V, 1J, 1D; X=V), whereas those of *D. sinobscura* show in diploid cells 12 chromosomes (2V, 1J, 2R, 1D; X=V). Interspecific matings between the two species were easy to obtain and produce fertile F_1 hybrids in both sexes.

Key words: Drosophilidae; *obscura* group; sibling species; China.

Introduction

China occupies a very important position when we consider the phylogeny and evolution of drosophilids distributed in the Northern Hemisphere, since adaptive radiations might have occurred in some genera, subgenera, species-groups or species- subgroups of various organisms in East Asia (THROCKMORTON, 1975, 1982)

Until recently, China was nearly a virgin territory regarding the *Drosophila obscura* species-group (BÄCHLI & ROCHA-PITÉ 1981; LAKOVAARA & SAURA, 1982). WATABE *et al.* (1996) described *Drosophila sinobscura* as a new species of this group from Taiwan, and reported about its distribution in the mainland of China, Hubei and Sichuan Provinces, too. It became conspicuous, however, that the continental strain of "*D. sinobscura*" derived from Hubei district could be reared easily under laboratory conditions whereas the insular strain from Taiwan proved less adaptive. We examined, therefore, the external morphology and the genitalia of individuals from the Hubei strain, and compared them to those of the type specimens of *D. sinobscura* WATABE. Further, karyotype

analyses were performed and sexual isolation was studied between these two strains, thought originally to be conspecific ones. The results indicate that these two allopatric strains of "*D. sinobscura*" differ from each other in a way that they should be considered as separated full species.

In the present article, we will describe the Hubei strain as a new species of the *D. obscura* group, followed by the results of karyotype analyses and crossing experiments. We will also discuss the evolutionary divergence of these two sibling species in relation to changes of paleoenvironments in the ranges of middle geographic latitudes of East Asia.

Materials and Methods

Rearing condition: The adult flies were allowed to oviposit in a glass vial (30 mm in diam., 105 mm in height) on *Drosophila* medium (10 ml), consisting of Baker's yeast (5 g)-sucrose (5 g)-cornmeal (5 g)-malt (5 g)-agar (0.8 g)-water (100 ml) mixtures with propionic acid (0.5 ml) added as an antiseptic. The rearing vials were maintained in incubators at 18±2℃ under continuous illumination.

Mating experiments: Sexual isolation was studied by no choice method, between six members of the *Drosophila obscura* subgroup: the present new species from Hubei (Hubei strain), *D. sinobscura* (Taiwan strain) and four other European species closely related to them. Newly emerged flies were slightly etherized for sex identification, and females and males were transferred into separate glass vials (25 mm in diam., 105 mm in height). The 8 to 12 day-old adult flies were used for all crosses. Five males and the same number of females were placed in a vial for 24 hrs. Then, females were dissected in Ringer solution and examined for sperm in seminal receptacles and spermathecae.

Karyotype observations: Preparations of mitotic chromosomes were made from neuroblasts of the 3rd instar larvae treated with 0.1 mg/ml of colchicine solution, and stained with 4% Giemsa solution (IMAI *et al.*, 1977; ASHBURNER, 1989). About 100 ganglion cells were examined for each species.

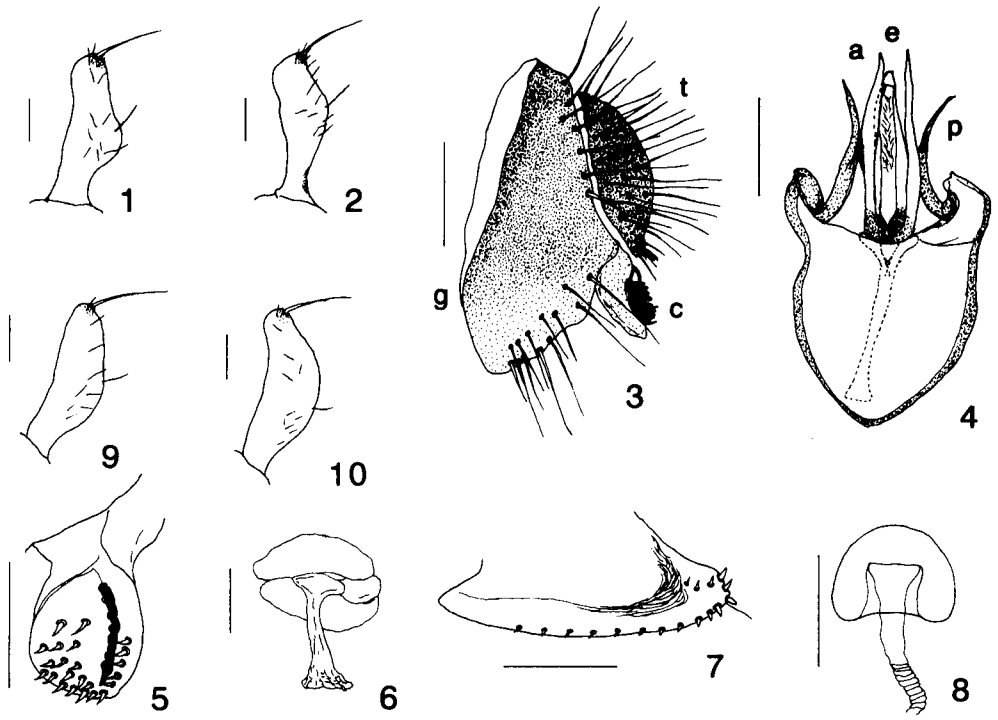
Results and Discussion

1. Taxonomic description

Drosophila (*Sophophora*) *hubeiensis* SPERLICH & WATABE sp. nov.

(Figs. 1–8)

♂, ♀. Head: Eye dark red with thick piles. Pedicel reddish brown, with



Figs. 1–10. *Drosophila* (*Sophophora*) *hubeiensis* (1–8) and *D. sinobscura* (9–10).—— 1 and 9, male palpus; 2 and 10, female palpus;3, male terminalia in lateral view; 4, aedeagus and its adjacent structures in ventral view; 5, inner aspects of surstylus; 6, ejaculatory apodeme;7, female ovipositor; 8, spermatheca. Signs: a, paramere; c, surstylus; e, aedeagus; g, epandrium; p, gonopods; t, cercus. Scale unit is 0.1 mm, except for in Figs. 5, 6 and 8 (0.05 mm).

a few stout setae; 1st flagellomere grayish brown. Frons black, anteriorly paler, *ca.* 0.42 as broad as head-width, with several inter-frontal setulae. Arista with 3 dorsal and 2 ventral branches besides terminal bifurcation. Anterior reclinate orbital setae (Orb2) *ca.* 0.46 length of posterior reclinate orbital setae (Orb1); proclinate setae (Orb3) *ca.* 0.81 length of Orb1. Gena brown, *ca.* 0.19 as broad as maximum diameter of eye. Subvibrissal setae (Or2) weak, *ca.* 0.39 length of vibrissae (Or1). Palpus with *ca.* 14 tiny setulae in ♂ and *ca.* 16 in ♀, in addition to 2 long setae at tip and on lateral margin (Figs. 1, 2).

Thorax: Scutum and scutellum black, without longitudinal stripes. Postpronotal lobe dark brown, with 2 long, stout setae (Hu). Anterior dorsocentral setae (DcA) *ca.* 0.74 length of posterior dorsocentral ones (DcP); length distance of dorsocentral setae *ca.* 0.42 cross distance. Acrostichal setulae (Ac) in 8 regular rows. Basal scutellar setae (SctB) nearly parallel; apical ones (SctA) heavily convergent. Anterior/posterior katepisternal seta length (Sterno- index) *ca.* 0.68.

Wing hyaline, slightly clouded. Veins dark brown; r–m and dm–cu

crossveins slightly fuscous. Wing indices: C *ca.* 2.79; 4V *ca.* 1.82; 4C *ca.* 0.94; 5x *ca.* 1.56; Ac *ca.* 2.33; C3F *ca.* 0.40. Halteres white, basally brown.

Legs dark brown; tibiae and tarsi paler. Large sex-combs present on metatarsus and 2nd tarsus of ♂ fore legs.

Abdominal tergites entirely black; ♂ 5th sternite (5S) pale brown, nearly rectangular, with tiny setulae.

Male terminalia (Figs. 3–6): Epandrium dark brown, paler on ventral half, with *ca.* 7 long setae along caudal margin and *ca.* 6 setae on ventral margin. Surstylus dark brown, distally with *ca.* 14 prensisetae and *ca.* 23 bristle-like setae on inner surface. Cercus hemispheric, ventrally tapering gently, with *ca.* 17 long setae and with several short ones on ventral apex. Aedeagus yellow, distally with tiny sensilla; aedeagal apodeme brown, apically pointed sharply; ejaculatory apodeme pale yellow, umbrella-shaped. Aedeagal guide black, rudiment. Paramere thin; gonopods bilobed basally. Hypandrium pale brown, without paramedian spines.

Female terminalia (Figs. 7–8): Oviscapt orange, roundish at caudal tip, with *ca.* 3 lateral and *ca.* 14 marginal ovisensilla, in addition to fine terminal sensillum. Spermatheca black, cone-shaped; introvert half of outer capsule height.

Holotype, ♂, China: Shennongjia (*ca.* 1800 m in alt.), Hubei Province, 27–28. vii. 1992, from timber piles (WATABE & TODA, leg); deposited in Institute of Zoology, Academia Sinica, Beijing, China (ASC).

Paratypes, China: 1 ♂, 2 ♀, same data as the holotype; 1 ♀ deposited in ACS and the remains in Biological Laboratory, Hokkaido University of Education, Sapporo, Japan (HUE).

Distribution. D. hubeiensis has been found only in highlands of Hubei and Sichuan Provinces, Central China.

Diagnosis. The new species belongs to the *obscura* subgroup, having long sex-combs on metatarsus and 2nd tarsus, 8 rows of Ac, and so on (STURTEVANT, 1942; WATABE *et al.*, 1996), and it closely resembles *D. sinobscura* and *D. subobscura* COLLIN. Among the characters examined so far, the chaetotaxy on palpus (Figs. 1–2, 9–10) and the number of prensisetae (Fig. 5) and of lateral ovisensilla (Fig. 7) are relatively reliable discernible characters for identifying the present species (WATABE *et al.*, 1996).

2. Morphological variation

Table 1 shows intra- and inter-specific variations of 31 quantitative characters of *D. hubeiensis* and *D. sinobscura* under the same laboratory condition as described above. As for the characters Nos. 1 to 4 and 8, the comparison was made for each sex separately. The range of all these characters overlapped interspecifically, although the interspecific differences proved statistically signifi-

Table 1. Intra- and inter-specific variations of quantitative characters in *D. hubeiensis* and *D. sinobscura*.

Quantitative character[1)]		*D. hubeiensis*			*D. sinobscura*			Interspecific difference(*t*-test)
		Mean±S. D.	Range	(*n*)	Mean±S. D.	Range	(*n*)	
1. Body length (mm)	♂	2.47±0.16	2.28–2.76	(20)	2.88±0.11	2.76–3.12	(10)	+ $p<0.01$
	♀	3.08±0.24	2.84–3.44	(20)	3.14±0.32	2.56–3.48	(10)	ns
2. Thorax length (mm)	♂	1.04±0.07	0.92–1.16	(10)	1.12±0.04	1.08–1.20	(10)	+$p<0.01$
	♀	1.24±0.07	1.12–1.32	(10)	1.26±0.05	1.20–1.32	(10)	ns
3. Wing length (mm)	♂	2.72±0.11	2.56–2.88	(10)	2.70±0.10	2.60–2.80	(10)	ns
	♀	3.20±0.10	3.08–3.40	(10)	3.24±0.21	2.76–3.52	(10)	ns
4. Wing width (mm)	♂	1.04±0.07	0.92–1.16	(10)	1.12±0.04	1.08–1.20	(10)	ns
	♀	1.24±0.05	1.12–1.28	(10)	1.25±0.05	1.16–1.32	(10)	ns
5. Frons width/head width		0.42±0.03	0.37–0.49	(20)	0.38±0.02	0.35–0.42	(20)	+$p<0.01$
Arista								
6. No. of upper branches		3.00±0.31	2–4	(42)	3.23±0.68	2–4	(44)	$\pm0.01<p<0.05$*[2)]
7. No. of lower branches		1.83±0.38	1–2	(42)	1.55±0.50	1–2	(44)	+$p<0.01$
8. Tiny setulae on palpus	♂	13.60±2.34	9–18	(30)	9.33±1.06	8–10	(30)	+$p<0.01$*
	♀	15.87±1.74	12–19	(30)	10.93±1.91	7–16	(30)	+$p<0.01$
9. Gena width/eye diameter		0.19±0.03	0.14–0.26	(20)	0.19±0.04	0.12–0.20	(20)	ns
10. Or2/Or1		0.39±0.09	0.25–0.67	(20)	0.40±0.09	0.20–0.50	(19)	ns
11. Orb2/Orb1		0.46±0.08	0.33–0.64	(20)	0.49±0.10	0.33–0.75	(19)	ns
12. Orb3/Orb1		0.81±0.13	0.55–1.13	(20)	0.85±0.14	0.60–1.13	(19)	ns
Thorax								
13. Lower/upper Hu length		0.99±0.11	0.76–1.17	(20)	0.96±0.23	0.40–1.33	(20)	ns*
14. DcA/DcP length		0.71±0.08	0.55–0.88	(20)	0.74±0.08	0.57–0.93	(20)	ns
15. Length distance/cross distance of Dc		0.44±0.06	0.33–0.54	(20)	0.42±0.06	0.33–0.59	(20)	ns
16. SctB/SctA length		0.94±0.08	0.83–1.13	(20)	1.00±0.11	0.80–1.33	(20)	$\pm0.01<p<0.05$
17. Distance from SctA to SctB/distance between SctAs		1.12±0.14	0.86–1.37	(20)	0.98±0.22	0.75–1.60	(20)	$\pm0.01<p<0.05$

Table 1. (Continued)

Quantitative character[1)]	*D. hubeiensis*			*D. sinobscura*			Interspecific difference(*t*-test)
	Mean±S. D.	Range	(*n*)	Mean±S. D.	Range	(*n*)	
18. Sterno-index	0.68±0.09	0.54–0.80	(20)	0.59±0.11	0.30–0.77	(19)	+p<0.01
Wing indices							
19. C	2.79±0.23	2.21–3.21	(20)	2.99±0.26	2.57–3.46	(20)	+p<0.01
20. 4V	1.82±0.18	1.39–2.25	(20)	1.80±0.16	1.50–2.07	(20)	ns
21. 4C	0.94±0.10	0.80–1.19	(20)	0.85±0.09	0.69–1.08	(20)	+p<0.01
22. 5x	1.56±0.19	1.14–1.83	(20)	1.64±0.23	1.14–2.00	(20)	ns
23. Ac	2.33±0.25	2.00–2.80	(20)	2.24±0.19	2.00–2.80	(20)	ns
24. C3F	0.40±0.05	0.32–0.50	(20)	0.37±0.07	0.22–0.50	(20)	ns
Sex-comb							
25. No. of teeth on metatarsus	10.53±1.07	8–14	(30)	8.83±1.21	7–11	(30)	+p<0.01
26. No. of teeth on 2nd tarsus	9.37±1.07	7–11	(30)	7.80±0.76	6–9	(30)	+p<0.01
27. No. of setae on ♂ 5S	14.45±1.46	11–16	(20)	16.65±1.42	14–19	(20)	+p<0.01
Epandrium							
28. No. of setae on upper half	6.10±0.91	4–7	(20)	5.95±0.89	5–8	(20)	ns
29. No. of setae on lower half	14.85±1.35	12–17	(20)	14.45±1.50	12–17	(20)	ns
Oviscapt							
30. No. of lateral ovisensilla	2.73±0.58	2–4	(30)	3.50±0.94	2–6	(30)	+p<0.01*
31. No. of marginal ovisensilla	13.47±1.01	12–15	(30)	15.27±1.17	13–18	(30)	+p<0.01

[1)] Abbreviations of quantitative characters are explained in the text.

[2)] Asterisks indicate Aspin-Welch method adopted in the case of unequal variance (*F*-test, $\alpha=0.01$).

Table 2. Percentages of inseminated females in the cross between *D. hubeiensis* and its related members of the *obscura* subgroup by no choice method. The numbers in parentheses give the number of females examined.

♂/♀	hub		sin		sub		amb		obs		tri	
hub[1)]	47.5	(101)	88.0	(101)	1.7	(121)	4.0	(100)	0.0	(77)	4.2	(120)
sin	37.9	(103)	90.5	(211)	2.8	(108)	2.0	(101)	1.0	(105)	4.8	(104)
sub	3.7	(108)	66.1	(116)	90.3	(144)	12.1	(66)	0.0	(63)	1.5	(60)
amb	0.0	(78)	6.9	(102)	0.0	(116)	81.5	(189)	1.0	(61)	9.7	(62)
obs	0.0	(58)	0.0	(106)	0.0	(62)	0.0	(62)	82.3	(124)	1.7	(68)
tri	0.0	(121)	32.5	(120)	1.5	(65)	4.3	(69)	0.0	(106)	83.5	(109)

[1)] hub, *D. hubeiensis*; sin, *D. sinobscura*; sub, *D. subobscura*; amb, *D. ambigua*; obs, *D. obscura*; tri, *D. tristis*.

Table 3. Influence of age and mating period to copulation in *D. hubeiensis*(hub) and *D. sinobscura*(sin).

	Fly age (day)	Mating period (hr)	Successful matings (%)
hub	8–12	24	47.5
	8–12	48	91.7
	16–20	24	44.0
	16–20	48	90.0
sin	8–12	24	90.5
	16–20	24	88.6

cant ($p<0.01$) in 14 (Nos. 1♂, 2♂, 5, 7, 8♂, 8♀, 18, 19, 21, 25–27, 30, 31) out of 36 comparisons. This means close resemblance of the two species in the external morphology and genitalia. Unless several kinds of quantitative characters are examined at the same time, a given specimen of either species cannot be identified unambiguously.

3. Studies on sexual isolation

Table 2 gives percentages of inseminated females in the matings between *D. hubeiensis* and five related species of the *D. obscura* subgroup by no choice method. In most species more than 80% of the females mated with their own conspecific males, but in *D. hubeiensis* only about half of the females did so. Among all interspecific crosses, the ratio of inseminated females was highest (88.0%) in the cross between *D. sinobscura* females and *D. hubeiensis* males, which attains the level of intraspecific crossings. Interspecific matings between *D. hubeiensis* and *D. sinobscura* abundantly produced progeny in both reciprocal crosses, and the F_1 hybrids were fertile in both sexes. The sex ratio (♀/♂) among newly emerged F_1 flies was nearly one; in the cross between *D. hubeiensis* females and *D. sinobscura* males the ratio was 1.06 ($n=200$) and 1.13 ($n=200$)

in the reciprocal cross. Any syndromes of hybrid breakdown were not observed in F_2.

Table 3 shows the dependence of fly age and the period of time given to pair on the insemination ratio of the two species *D. hubeiensis* and *D. sinobscura*. About 90% of *D. hubeiensis* females mated with their males in 48 hrs but less then 50% in 24 hrs. However, the relative low percentage ratio of successful matings in the 24 hrs experiments was probably not due to inpersistance in courtship activity of their males but rather to a high threshold of acceptance of *D. hubeiensis* females.

It is notable that the percentages of successful matings are quite different among the three closely related species, *D. hubeiensis*, *D. sinobscura* and *D. subobscura*. Only 3.7% of *D. hubeiensis* but 66.1% of *D. sinobscura* females accepted *D. subobscura* males. Further, *D. hubeiensis* females did not accept *D. tristis* males at all, but one third of *D. sinobscura* females mated with these latter males. Such differences in interspecific mating preferences also reflect differentiation of courtship behaviors of *D. hubeiensis* and *D. sinobscura*.

Interspecific crossings under laboratory conditions are quite rare for most species pairs of European members of the *D. obscura* group, and if F_1 males are produced they are normally completely or at least partially sterile. Just the same was observed for a pair of North American sibling species of the *pseudoobscura* subgroup, *D. pseudoobscura* FROLOVA and *D. persimilis* DOBZHANSKY & EPLING (DOBZHANSKY & POWELL, 1975). Our results, a high hybridization compatibility and a morphologically close resemblance, assert that *D. hubeiensis* and *D. sinobscura* should be placed at levels of subspecies if not conspecific strains.

4. Karyotypes

The karyotypes of *D. hubeiensis* and *D. sinobscura* differ from each other clearly and are therefore fully diagnostic (Figs. 11a–b). The male metaphase configuration of *D. hubeiensis* shows a diploid number of $2n=10$, consisting of 3 pairs of metacentric (V-shaped), 1 pair of submetacentric (J-shaped) and 1 pair of dotlike chromosomes (D). Both X and Y chromosomes are metacentric. This chromosome complement of $2n=10$ (3V, 1J, 1D) resembles that of three European relatives (4V, 1D), *D. ambigua* POMINI, *D. tristis* FALLÉN, and *D. eskoi* LAKOVAARA & LANKINEN and of the Palearctic species *D. bifasciata* POMINI (LAKOVAARA & SAURA, 1982). On the other hand, all cells of *D. sinobscura* show a diploid number of $2n=12$, with 2 pairs of metacentrics, 1 pair of submetacentrics, 2 pairs of acrocentrics (Rod-shaped) and 1 pair of microchromosomes. Both X and Y are metacentric. This karyotype with the highest number of chromosomes in the *D. obscura* species-group (2V, 1J, 2R, 1D), is similar to that of *D. obscura* FALLÉN (3V, 2R, 1D), an European member and

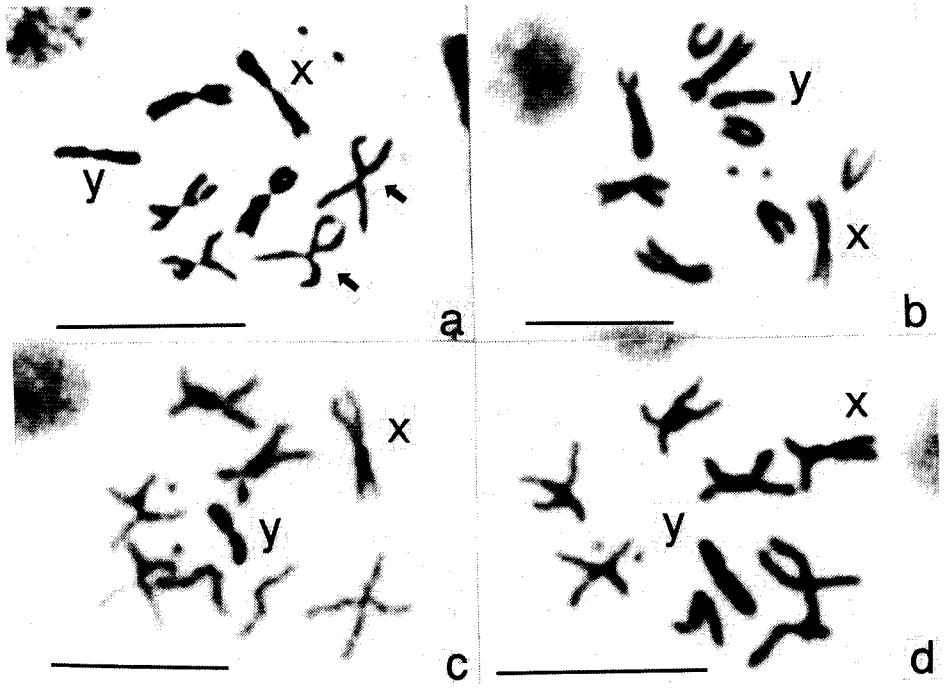


Fig. 11. Male metaphase chromosomes of *D. hubeiensis* (a), *D. sinobscura* (b), F_1 hybrid larva between *hubeiensis* ♀ and *sinobscura* ♂ (c), and F_1 hybrid larva between *sinobscura* ♀ and *hubeiensis* ♂ (d). Scale unit is 10 μm.

of *D. tsukubaensis* TAKAMORI & OKADA, a Japanese endemic species of the *obscura* subgroup. One pair of the V- shaped chromosomes of *D. hubeiensis* is very large (with arrows in Fig. 11a), being about twice as long as 2 pairs of acrocentric autosomes of *D. sinobscura.* Thus, differences in the chromosome number between them might be explained by centric fusion of 2 pairs of acrocentric autosomes found in *D. sinobscura*, or by centric fission of 1 pair of metacentric autosomes in *D. hubeiensis.* The reduction in chromosome number is likey to be a frequent trend in the family Drosophilidae, since the formation of two rod-shaped chromosomes from a V-shaped one demands an additional centromere (CLAYTON & GUEST, 1986). Comparing the situation with that of other species of the *D. obscura* group the former hypothesis of centric fusion appears much more probable than the latter.

Figures 11c and 11d show the metaphase plate of F_1 hybrid males from an inter-cross between *D. hubeiensis* females and *D. sinobscura* males and from the reciprocal cross, respectively. In both hybrids the number of chromosomes is $2n=11$ (5 metacentric, 2 submetacentric, 2 acrocentric and 2 micro-chromosomes). These configurations clearly prove that the karyotype of F_1 hybrids is derived from gametes of both parents.

5. Evolutionary history and species divergence

As shown above, *D. sinobscura* and *D. hubeiensis* are very similar morphologically. They can be easily inter-crossed and yield fertile offspring in both sexes. No sexual isolation exists between members of the two species. However, they behave differently, when mated with other closely related European species, *D. subobscura* and *D. tristis* (Table 2). In addition to the difference in the chromosomal karyotype, the two are also different in allozyme divergence, DNA differentiation of sat DNA sequences, and alcohol dehydrogenase gene (WATABE *et al*, 1997), suggesting two distinct sibling species of the *D. obscura* group.

In the evolutionary sense, the present biogeographic pattern of the *obscura* species-group appears to reflect the influence of alternative paleoenvironments. It is difficult to trace the evolutionary process of *D. hubeiensis* and *D. sinobscura* with accuracy, without enough knowledge about the Chinese relatives and fossil record in this group. Some limit evidences on the paleogeography, however, apparently exist when inferring the evolutionary history. The *obscura* group undoubtedly is a temperate-forest species of the Northern Hemisphere. Flies of this group inhabit both lowland forests and mountains in cool temperate region (e.g., Hokkaido Is.), but they are restricted to mountains only in warm temperate areas (e.g., Kyushu Is.). Consequently, *D. hubeiensis* and *D. sinobscura* are found in the subtropical regions of China only in highlands with elevations of more than 1,200 meters. As discussed previously (WATABE *et al.*, 1993, 1996), it is reasonable to assume that a hypothetical ancestor common to this two species and also to *D. subobscura*, might have lived from Oligocene to early Miocene when temperate forests were widely distributed in middle geographic latitudes of Eurasia (TANAI, 1971; THROCKMORTON, 1975). Later, a huge arid zone began to develop in Central Asia at same latitudes. Geographic isolation appears to have played an important role in the evolution of *Drosophila* (MAYR, 1974), and the Central Asia deserts might have favored speciation processes in the ancestor populations leading to the species of the *obscura* subgroup that are endemic now to Europe or East Asia.

Evience on the morphology, karyotype and sexual isolation we have presented clearly demonstrates that *D. hubeiensis* and *D. sinobscura* are much closer to each other than any other species pair in the *obscura* species-group, suggesting that the split from a common ancestor may be a more recent event on a geological time scale. Active orogenesis in the Taiwan Central Range started from late Pliocene to mid Pleistocene (HO, 1967), and the depth of the Taiwan Straits is less than 100 meters even today. Taiwan is, therefore, considered as a peripheral district of Eurasia during the Riss and Würm Glacial Ages, when the annual mean temperature was lower than at present time by an average of about 8–10℃ and the sea level was lower by about 100–130 meters

(LIN, 1963; TING-YING, 1964; ZHENG *et al.*, 1994). Based on studies on grain size distribution, XIAO *et al.* (1992) showed that winter monsoons became strongest in the Loess Plateau of Central China (north-west from Hubei Province), about 18,000 years ago, during the Riss Ice Age. Under such severe climates, East Asia provided old Tertiary temperate elements leaving refuges from the Great Ice Age of the Pleistocene in the mountains. In fact the well-known living fossils, *Metasequoia glyptostoroboides* HU & CHENG and *Ginkgo biloba* L., have been discovered in some mountains of Hubei and Sichuan Provinces. It is probable that the pre- "*hubeiensis-sinobscura*" ancestor might have been spread widely in middle latitudes of East Asia including Taiwan during the glacial age. With warming up of climate and the opening of the Taiwan Channel about 10,000 years ago, some of the *obscura* group flies retreated northwards, whereas others survived in highlands with lower temperatures. Since then, gene exchange between continental and insular populations of the common ancestor was probably terminated, leading to the final split between *D. hubeiensis* and *D. sinobscura.*

Recent molecular studies have produced data for the investigation of the phylogenetic relationships of *Drosophila* species. Based on restriction site analysis of mtDNA, LATTORRE *et al.* (1988) estimated the time of split between the *obscura* subgroup species endemic to Eurasia and the *pseudoobscura* subgroup species endemic to North America as 6 Mya. Studying the sequences of alcohol dehydrogenase genes, RUSSO *et al.* (1995) estimated the time as 13 Mya. Both values fall in the Miocene age when the temperate deciduous forests developed at high latitudes of the Northern Hemisphere and when some species that originated in Eurasia spread to North America, most probably by way of Beringia (THROCKMORTON, 1975). The specific split within each subgroup happened at later periods. The origin of the present *Drosophila obscura* species-group is still an open question, and faunal studies have just started in China. Further biogeographic, geological and genetic investigations of Chinese drosophilids are indispensable for evaluating a real picture of the evolution of the *obscura* species-group.

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