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

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Abstract A new species of the obscura species-group from China, Drosophila (Sophophora) sinobscura, is described together with the results of sexual isolation studies. The present species closely resembles D. subobscura, an European member of the obscura subgroup, in the external morphology and genitalia, and a high percentage of successful matings was observed between these two allopatric species.

Key words: Drosophilidae; Drosophila; obscura group; China.

Introduction

The Drosophila obscura species-group involving about thirty species has extensively been studied in the field of evolutionary biology (DOBZHANSKY & POWELL, 1975; LAKOVAARA & SAURA, 1982; GONZÁLEZ et al., 1990, etc.). Most species are distributed in cool temperate and cold territories of the Holarctic region, and six members are presently known from East Asia, Japan and Korea. As LAKOVAARA and SAURA (1982) have already stated, there is a huge gap in our knowledge on the geographic distribution of the group in China and Siberia (BÄCHLI & ROCHA PITÉ, 1981).

We have recently found a new species of the obscura group in the mainland of China and Taiwan, which is more similar to some European relatives, especially D. subobscura, than Asiatic and American ones in the external appearance. The present paper deals with a description of Drosophila (Sophophora) sinobscura sp. nov., and discusses its systematic relationships from the results of morphological comparison and sexual isolation studies.

Materials and Methods for Cross Experiments

Sexual isolation was studied between the present new species and four

European representatives of the obscura species-subgroup, D. obscura Fallén, 1823, D. tristis Fallén, 1823, D. subobscura Collin (in Gordon, 1936) and D. ambigua Pomini, 1940. An iso-female line of the new species was used, which was collected at Chitou, Taiwan, in mid March of 1992. All of the European strains were provided by Prof. D. Sperlich (University of Tübingen). The degree of sexual isolation was studied by two cross methods, no choice and male choice methods.

In no choice method, newly emerged flies were slightly etherized and sorted for sex every day, and were stored in glass vials (40 mm in diam., 120 mm in height) with malt-yeast medium at 20°C. The 8 day-old to 12 day-old adult flies were used for all crosses. Five males and the same number of alien females were placed together in a vial for 24 hrs, and the females were dissected in Ringer solution and examined for sperm in seminal receptacles and spermathecae. About 12 to 40 replicates were made for the crosses.

In male choice method, five males were placed together with ten females, five owns and five aliens, in a vial. The females were examined for sperm after 24 hrs. Fifteen replicates were made for each cross. The degree of sexual isolation was evaluated by the STALKER's isolation index (1942).

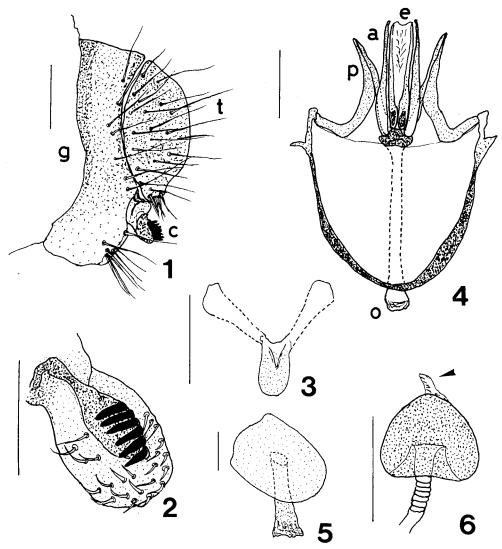
Drosophila (Sophophora) sinobscura WATABE, sp. nov.

(Figs. 1-6, 7A-9A)

 \mathcal{T} , \mathcal{T} . Head: Eye dark red with thick piles. Pedicel reddish brown, with a few setae; 1st flagellomere grayish brown. Frons black, anteriorly paler, 0.38 (n=20); range 0.35–0.42) as broad as head-width, with several interfrontal setulae. Arista with 3 (2–3) dorsal and 2 (1–2) ventral branches besides terminal bifurcation. Anterior reclinate orbital setae (Orb 2) 0.49 (0.42–0.75) length of posterior reclinate orbital setae (Orb 1); proclinate setae (Orb 3) 0.86 (0.67–1.00) length of Orb 1. Face dark brown; facial carina broad; clypeus black. Gena brown, 0.19 (0.12–0.25) as broad as maximum diameter of eye. Subvibrissal setae weak, 0.41 (0.13–0.50) length of vibrissae.

Thorax: Scutum and scutellum black, without longitudinal stripes. Post-pronotal lobe dark brown, with ca. 2 setae. Anterior dorsocentral setae 0.74 (0.57–0.93) length of posterior dorsocentral ones; length distance of dorsocentral setae 0.42 (0.33–0.59) cross distance. Acrostichal setulae in 8 regular rows. Sterno-index 0.58 (0.30–0.77).

Wing hyaline, slightly clouded. Veins dark brown; r-m and dm-cu crossveins slightly fuscous. R_{2+3} gently curved to costa at tip; R_{4+5} and M nearly parallel. Wing indices: C=2.83 (2.57–3.36) in \nearrow , 3.16 (2.93–3.46) in ?; 4V=1.80 (1.50–2.07); 4C=0.85 (0.69–1.08); 5x=1.79 (1.50–2.00) in \nearrow , 1.48 (1.14–1.67) in ?; Ac=2.24 (2.00–2.80); C3F=0.37 (0.22–0.50). Halteres white,



Figs. 1-6. Drosophila (Sophophora) sinobscura WATABE, sp. nov. 1, Male terminalia in lateral view; 2, inner aspects of surstylus; 3, decasternum; 4, aedeagus and its adjacent structures in ventral view; 5, ejaculatory apodeme; 6, spermatheca. Signs: a, paramere; c, surstylus; e, aedeagus; g, epandrium; o, aedeagal apodeme; p, gonopods; t, cercus. Scales=0.05 mm, except for in Figs. 1 and 4 (0.1 mm).

basally brown.

Legs dark brown; tibiae and tarsi paler. Large sex combs present on metatarsus and 2nd tarsus of \mathcal{I} fore legs.

Male terminalia (Figs. 1-5): Epandrium dark brown, paler on ventral half, with ca. 7 long setae along posterior margin and ca. 6 setae on ventral margin. Surstylus black, with ca. 6 prensisetae and ca. 23 bristle-like setae. Cercus hemispheric, ventrally tapering gently, with ca. 17 long setae, and with several setulae on ventral apex. Decasternum Y-shaped, less-sclerotized. Aedeagus

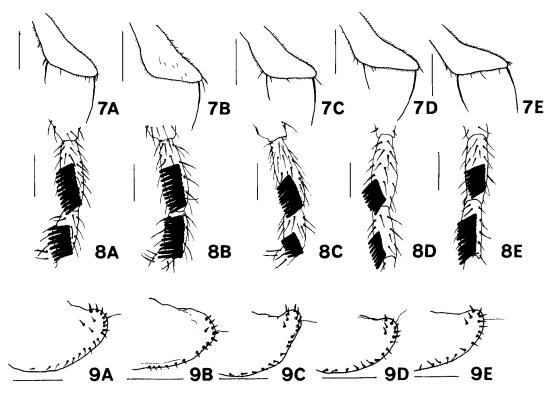
yellow, distally with tiny sensilla; aedeagal apodeme brown; ejaculatory apodeme light brown, umbrella-shaped. Aedeagal guide black, rudiment. Paramere thin; gonopods bilobed basally. Hypandrium pale brown, without paramedian spines.

Female terminalia (Figs. 6, 9A): Oviscapt orange, roundish at caudal tip, with ca. 3 lateral and ca. 18 marginal ovisensilla in addition to fine terminal sensillum. Spermatheca black, cone-shaped; introvert shallow, ca. 2/5 height of outer capsule; some specimens with apical projection (arrow in Fig. 6).

Measurements: Body length = 2.87 mm (n = 10; range: 2.76-3.12) in \mathcal{S} , 3.14 mm (n = 10; 2.56-3.48) in \mathcal{S} . Thorax length including scutellum = 1.12 mm (1.08-1.20) in \mathcal{S} , 1.26 mm (1.20-1.32) in \mathcal{S} . Wing length = 2.70 mm (2.60-2.80) in \mathcal{S} , 3.24 mm (2.76-3.52) in \mathcal{S} , and its width = 1.08 mm (1.00-1.20) in \mathcal{S} , 1.25 mm (1.16-1.32) in \mathcal{S} .

Pre-adult stages: Eggs with two filaments. Larvae tend to pupate either on or just above the surface of the medium in a culture vial.

Holotype, ♂, China: Chitou (ca. 1140 m in alt.), Taiwan Province, 10. iii. 1992, ex. banana trap (WATABE leg.); deposited in Biological Laboratory, Hokkaido University of Education (HUE), Sapporo, Japan.



Figs. 7-9. Some diagnostic characters of D. sinobscura (A) and its allied species of the obscura subgroup, D. subobscura (B), D. ambigua (C), D. obscura (D) and D. tristis (E).
7, Chaetotaxy on palpus; 8, sex combs on male forelegs; 9, female terminalia. Scales = 0.1 mm.

Paratypes, China: $5 \nearrow$, 5 ?, same data as holotype; $3 \nearrow$, 3 ? in Institute of Zoology, Academia Sinica, Taipei, China, and the remaining paratypes in HUE.

Distribution. D. sinobscura has been recorded from the Oriental region of China, and it inhabits highlands with relatively cool climates. The specimens examined this time are as follows: $2 \checkmark$, $2 \textdegree$, Shennongjia (ca. 1800 m), Hubei Province, 27–28. vii. 1992, from timber piles (WATABE & TODA leg.); $3 \textdegree$, Mt. Emei (ca. 2000 m), Sichuan Province, 19. vii. 1992, ex. banana traps (WATABE leg.).

Relationships and diagnosis. D. sinobscura belongs to the obscura subgroup, having broad carina, eight rows of Ac, large sex-combs on the male foreleg, and so on (STURTEVANT, 1942; LAKOVAARA & SAURA, 1982; CARIOU et al., 1988). D. sinobscura closely resembles D. subobscura in the external morphology (e.g., hyaline wing, females lacking yellow spots on the abdominal tergites), in the genitarial structure (e.g., number of teeth on surstylus) and in the pupation behavior mentioned above, but the former can be distinguished from the latter by the chaetotaxy on palpus (Figs. 7A, B). Further, D. sinobscura is distinguishable from D. ambigua by the shape of ovipositor (Figs. 9A, C), from D. obscura by the length of sex-comb (Figs. 8A, D), and D. tristis by the chaetotaxy on palpus (Figs. 7A, E) and by wing coloration (cf. Bächli & Burla, 1985).

Etymology. The specific epithet is a Chinese member of the obscura species-group.

Sexual Isolation

Table 1 gives percentages of inseminated females within the obscura subgroup by no choice method. More than 80% of the females mated with their own males in every cross, whereas the successful mating was limited in most of interspecific crosses. Of all crossings, the ratio of inseminated females was highest (66.1%) in the cross between D. sinobscura females and D. subobscura males but it was extremely low in its reciprocal cross (2.8%). The matings were quite limited between D. sinobscura females and males of other species, being

| Table 1. Percentages | of inseminated females in the cross between obscura subgroup species by |
|----------------------|---|
| no choice method. | The numbers in parentheses give the number of females examined. |

| <u>우</u> | D. sinobscura | D. subobscura | D. ambigua | D. obscura | D. tristis |
|---------------|---------------|---------------|------------|------------|------------|
| 8 | | | | | |
| D. sinobscura | 90.5 (211) | 2.8 (108) | 2.0 (101) | 1.0 (105) | 4.8 (104) |
| D. subobscura | 66.1 (115) | 90.3 (144) | 12.1 (66) | 0.0 (63) | 1.5 (60) |
| D. ambigua | 6.9 (102) | 0.0 (116) | 81.5 (189) | 1.0 (61) | 9.7 (62) |
| D. obscura | 0.0 (106) | 0.0 (62) | 0.0 (62) | 82.3 (124) | 1.7 (58) |
| D. tristis | 32.5 (120) | 1.5 (65) | 4.3 (69) | 0.0 (106) | 83.5 (109) |

Table 2. Sexual isolation between *D. sinobscura* (SI) and *D. subobscura* (SU) in the cross by male choice method. Homogamic (+) and Heterogamic (+) indicate the number of females inseminated by their own males and by alien males, respectively. Isolation index (I.I.) = [% of Homogamic (+) - % of Heterogamic (+)]/
[% of Homogamic (+) + % of Heterogamic (+)].

| Crosses | | Number of Homogamic | | Number of Heterogamic | | % of Homogamic | | % of Heterogamic | | I.I. |
|---------|----|------------------------|----|--------------------------|----|----------------|------|------------------|-------|------|
| | | | | | | | | | | |
| SI + SU | SU | 49 | 26 | 40 | 39 | 65.3 | 34.7 | 50.6 | 49.4 | 0.13 |
| SI + SU | SI | 50 | 25 | 0 | 75 | 68.5 | 31.5 | 0.0 | 100.0 | 1.00 |

32.5%, 6.9% and 0.0% in the crosses with *D. tristis*, *D. ambigua* and *D. obscura* males, respectively. Males of *D. sinobscura* were also rejected by the females of these three species.

Table 2 shows the results of sexual isolation in crosses by male choice method. In the cross of (*D. sinobscura* $\stackrel{\circ}{+} + D$. subobscura $\stackrel{\circ}{-} + D$. subobscura $\stackrel{\circ}{-} + D$. subobscura $\stackrel{\circ}{-} + D$. subobscura the isolation index was only +0.13, being statistically insignificant in the χ^2 test ($\alpha = 0.05$), which means an incomplete sexual interruption between two species. We examined 200 eggs laid by *D. sinobscura* females with *D. subobscura* males but did not observe any syndrome of egg development.

An asymmetric mating preference, which is presently detected in the cross between *D. sinobscura* and *D. subobscura*, has been frequently reported in the cross between closely related species or subspecies of *Drosophila* (WATANABE & KAWANISHI, 1979; KANESHIRO, 1980; WATABE et al., 1990). The present distribution of *D. subobscura* extends eastward to the Tianshan Mountains in Central Asia but *D. sinobscura* has not been found there (WATABE et al., 1993), which shows that they are geographically isolated from each other by a huge arid zone of the Gobi and Tacklimakan Deserts developing in middle latitudes of Central Asia.

The obscura group is strongly sylvicolous, and *D. sinobscura* dwells in highlands with a relatively cool climate. *D. sinobscura* might have arisen from ancestor common to *D. subobscura*, probably from Oligocene to early Miocene when temperate forests would well spread in middle latitudes of Eurasia with the decreasing of temperature (TANAI, 1971; THROCKMORTON, 1975).

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