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Two New Synonyms of *Drosophila tsigana* BURLA et GLOOR (Diptera, Drosophilidae), with the Results of Cross-Experiments

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Abstract *Drosophila pengi* OKADA et KUROKAWA 1957 and *Nesiodrosophila septentriata* TAKADA et MAEKAWA, 1984 are newly synonymized with *D. tsigana* BURLA et GLOOR, 1952, based on the morphological comparison and the results of cross-experiments.

Introduction

Up to the present, six species of the *Drosophila melanica* species-group have been reported from the Old World: *D. tsigana* BURLA et GLOOR, 1952 from Western Europe (the Pyrénées Mountain Range and its adjacent districts), *D. longiserrata* TODA, 1988 and *D. bisetata* TODA, 1988 from Burma, *D. afer* TAN, HSU et SHENG, 1949 from China, *D. pengi* OKADA et KUROKAWA, 1957 from Japan and Korea, and *D. moriwakii* OKADA et KUROKAWA, 1957 from Japan. The last species was recently transferred from the *D. robusta* group to the *D. melanica* group by BEPPU (1988).

According to the description by BURLA and GLOOR (1952), referring to the genitalia briefly, *D. tsigana* seems to be very close or conspecific with *D. pengi*. Further, another Japanese species, *Nesiodrosophila septentriata* TAKADA et MAEKAWA, 1984, is very similar to these two species. We had an opportunity to examine *D. tsigana* by courtesy of Dr. BÄCHLI (University of Zürich), in comparison with the two Japanese species, and concluded that all the three are conspecific. This paper provides the redescription of *D. tsigana*, especially for the genitalia, and reports the results of cross-experiments, with special reference to the morphological and genetical differentiation between the western and eastern populations distributed at the opposite ends of the Palearctic Region.

Materials and Methods for Cross-Experiments

The degree of reproductive isolation was studied between European (E) and Japanese (J) populations of *D. tsigana* by two cross-methods.

Two culture strains representing the following local populations were used: E) Ariège (42°55′ N, 1°10′ E), St. Girons, France, established from wild flies caught in 1985; J) Misumai (42°57′ N, 141°16′ E), Hokkaido, Japan, established in 1988.

No choice method. Newly emerged flies were etherized and sorted for sex every day, and were stored in vials (40 mm in diam., 120 mm in height) with usual *Drosophila* medium at 20°C under continuous light. As *D. tsigana* is a slow breeder, the 16-day-old flies were used for all crosses. Ten males and ten alien females were placed together in a vial for two days, and the females were dissected in RINGER solution and examined for sperm in both spermathecae and seminal receptacles. Eight replicates were made for each cross. In addition, the degree of emergence of hybrids was checked.

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 two days. Six replicates were made for each cross. The degree of sexual isolation was evaluated by the STALKER's isolation index (1942).

Drosophila (*Drosophila*) *tsigana* BURLA et GLOOR

(Figs. 1–7)

Drosophila tsigana BURLA et GLOOR, 1952, Z. indukt. Abstammungs- und Vererbung., **84**: 164.
Drosophila (*Drosophila*) *pengi* OKADA et KUROKAWA, 1957. Kontyû, Tokyo, **25**: 11. **N. syn.**
Drosophila melanissima. KIKKAWA & PENG, 1938, Jpn. J. Zool., **7**. 338 (nec SIUKIEVANI, 1916).
Nesiodrosophila septentriata TAKADA et MAEKAWA, 1984, J. Fac. gener. Educ., Sapporo Univ., (25): 42. **N. syn.**

General external morphology and chaetotaxy of *D. tsigana* were offered in the above papers, and its genitalia are redescribed as follows.

Periphallic organs (Figs. 2–3): Epandrium posteriorly pubescent, with ca. 9 long bristles on ventral portion. Cercus oval, fused to epandrium, with ca. 28 long bristles and tuft of several short bristles along ventral margin. Surstylus rectangular, pubescent except proximal portion, with ca. 13 primary teeth and ca. 7 bristles.

Phallic organs (Figs. 4–5): Aedeagus straight, roundish on distal margin, separated into two lateral lobes, which fused only at tip; apodeme as long as aedeagus; vertical rod absent. Anterior paramere rod-shaped; posterior one absent. Hypandrium pubescent, with 1 pair of submedian spines on anterior portion. Ventral fragma narrow, arc-shaped.

♀ reproductive organs (Figs. 6–7): Lobe of ovipositor slender, with ca. 3 discal and ca. 30 marginal teeth. Spermatheca nearly quadrate, wrinkled on basal

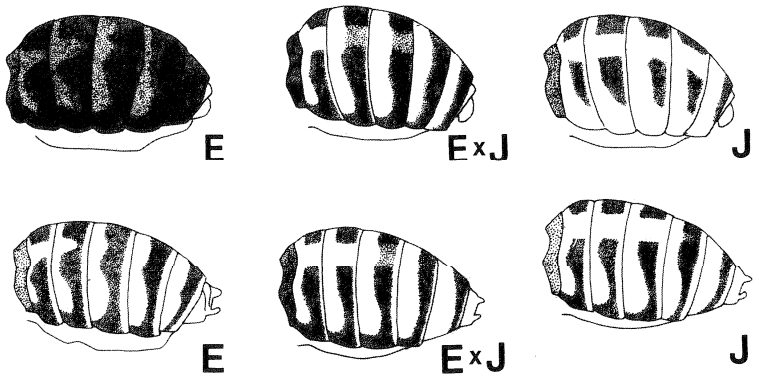

Fig. 1. Abdominal color patterns of European (E) and Japanese (J) populations of *Drosophila tsigana* and their hybrids (E × J), reared under continuous light at 20°C. Upper: ♂, Lower: ♀.

half of outer capsule, without apical indentation; introvert ca. 3/5 of height of outer capsule.

Distribution. Western Europe, Korea, Japan.

Geographical variation. Some morphological, but slight, differences are seen between E and J populations. Second to 5th tergites of J male have small black caudal bands interrupted at middle, whereas those of E male are almost entirely black (Fig. 1). In female, E has broader caudal bands on tergites than J. It is known that in many drosophilid species body color is more or less changeable according to culturing temperatures (*e.g.*, WATABE, 1977). However, the above difference in the abdominal color pattern between E and J is relatively constant, *i.e.*, E is always darker than J, if both are cultured at the same temperature. Moreover, the following slight differences on the genitalia were detected: all organs excepting spermatheca paler in J (Figs. 2 B–6 B); epandrium pubescent slightly more narrowly in J (Fig. 2 B); spermatheca slightly constricted submedially in J (Fig. 7 B); epandrium somewhat pointed ventrally (Fig. 2 B) and aedeagus slightly projected apically (Fig. 4 B) and broaden in lateral view (Fig. 5 B) in some specimens of J. The shapes of epandrium and aedeagus are nearly constant in E (Figs. 2 A, 4 A, 5 A), but vary continuously in J; one extreme case is shown in Figs. 2 B, 4 B and 5 B, and the other extreme is nearly identical to E's type.

Cross-Experiments

In no choice method, E and J mated well to each other in both crosses (Table 1). In these crosses hybrid flies emerged abundantly for both sexes, and produced the fertile F_2 offspring. The abdominal color patterns of F_1 flies were almost

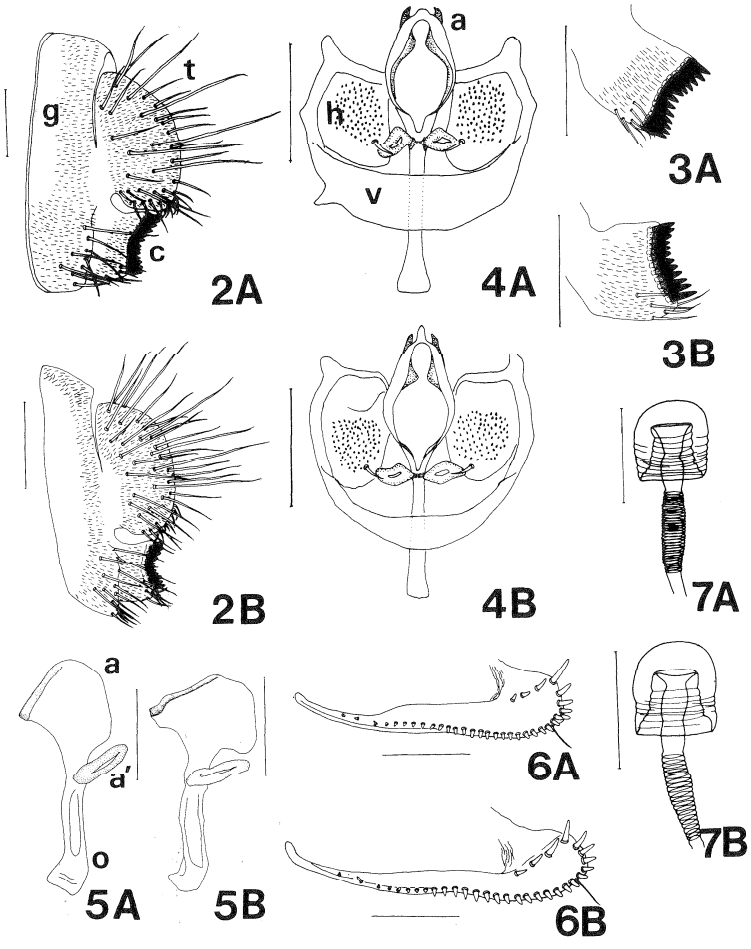

Fig. 2–7. Male and female genitalia of European (A) and Japanese (B) specimens of *Drosophila tsigana*. —— 2, Periphallic organs; 3, surstylus; 4, phallic organs; 5, aedeagus (in lateral view); 6, ovipositor; 7, spermatheca. Signs: a, aedeagus; a′, anterior paramere; c, surstylus; g, epandrium; h, hypandrium; o, apodeme; t, cercus; v, ventral fragma. Scale-line=0.1 mm.

intermediate between the parental ones, and were quite variable in the following generation (Fig. 1).

Table 2 gives the results of the sexual isolation in crosses by male choice method. In the cross of E♀ +J♀/J♂ the isolation index was +0.35, being statistically significant in the χ^2 test (α=0.01), which means that J males prefer to mate to its own females. In the cross of E♀ +J♀/E♂ the value was −0.01,

Table 1. Percentages of inseminated females in the crosses between European (E) and Japanese (J) populations of *Drosophila tsigana* by no choice method.

♀ \ ♂	E	J
E	85.0 (80)	77.6 (49)
J	85.7 (63)	83.7 (76)

The numbers in parentheses give the number of females examined.

Table 2. Sexual isolation between European (E) and Japanese (J) populations of *Drosophila tsigana* in the crosses by male choice method.

Cross			Homogamic		Heterogamic		% of Homogamic		% of Heterogamic		I.I.
Female		Male	(+)	(−)	(+)	(−)	(+)	(−)	(+)	(−)	
E	J	E	50	9	50	8	84.7	15.3	86.2	13.8	−0.01
E	J	J	45	15	22	39	75.0	25.0	36.1	63.9	0.35

Homogamic (+) and Heterogamic (+) indicate the number of females inseminated by their own males and by alies males, respectively. Isolation Index (I.I.)=[% of Homogamic (+)−% of Heterogamic (+)]/[% of Homogamic (+)+% of Heterogamic (+)].

which indicates no sexual interruption between the strains.

Thus, the results of cross-experiments, free crossings and fertile hybrids for both sexes, strongly support our conclusion based on the morphological comparison.

An asymmetric mating preference has been frequently reported in the cross between closely related species (WATANABE & KAWANISHI, 1979, 1981). The asymmetric crossability detected in the crosses by male choice method, as well as the morphological variations, may reflect a genetical divergence between European and Japanese populations. *Drosophila tsigana* is supposed to have emerged in a temperate forest of the East Asia and then to have spread to western parts of the Old World (THROCKMORTON, 1975). It is, however, unkown whether its present distribution range is continuous from Europe to Japan or interrupted widely in the middle part, because of the lack of information from Central Asia. If the morphological variations would be discontinuous there, these E and J forms each might be regarded as a subspecies of *D. tsigana, i.e., D tsigana tsigana* for the former and *D. tsigana pengi* for the latter.

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