

**Note on the blood sources of major two  
*Prosimulium* species in Hokkaido,  
Japan (Diptera : Simuliidae)<sup>1)</sup>**

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**Introduction**

Black flies (Simuliidae) are well known to be important pests to both human and domestic animals because of their blood feeding behaviour. Many effects of medico-veterinary and economic importance of black flies are described by many authors 2), 3), 4), 5), 6), 15), 17). The main effects are the transmission of various parasites and pathogenic micro-organisms such as human and bovine onchocerciasis and avian leucocytozoonosis. Therefore, some studies on the blood source animals for black flies have been reported in Japan; most of them are based on the data from human attractant or various animal-baited traps 7), 8). The authors have previously reported the blood source animals of black flies collected at the Onnebetsu-dake area determined by the use of the enzyme-linked immunosorbent assay (ELISA) 14), and the study is moreover the only one which used immunological techniques to determine the blood source of black flies in Japan.

Despite that more than thirty species belonging to thirteen genera of family Simuliidae are known from Hokkaido 10), 12), a few species have been known their blood source animals, and most of the knowledges are based on the field observations.

In the present paper, the authors describe the blood source animals of two major *Prosimulium* species of Hokkaido, namely *Prosimulium jezonicum* (Matsumura, 1931) and *P. yezoense* Shiraki, 1935, determined by the use of ELISA.

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## Materials and Methods

### 1. Preparation of blood-meal specimens

Black flies were collected with human attractant, sweeping method from surrounding of pasturing cattle and ground vegetations and shrubs and CO<sub>2</sub>-lured mosquito-net traps in several localities in Hokkaido (Fig. 1 & 2) during

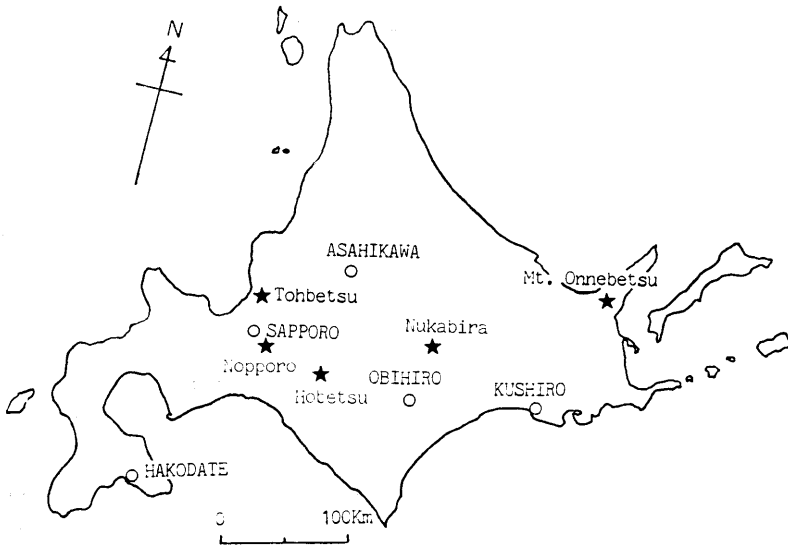


Fig. 1. Map of Hokkaido ★ show collecting localities of *P. jezonicum*.



Fig. 2. Map of Hokkaido ★ show collecting localities of *P. yezoense*.

the occurring periods during from 1984 to 1986. The collected black fly specimens were put in an ice box and transported to the laboratory for identification. The identified specimens were pressed onto a filter paper (Toyo No. 1) individually. The smeared filter papers were dried rapidly with diphosphorus pentoxide in a desiccator, then smeared parts were cut off with scissors and stored individually in micro-vials until assay.

## 2. Preparation of antiserum

The antisera used in this study were anti-wild brown bear (*Ursus arctos yesoensis*) serum, anti-sika deer (*Cervus nippon yesoensis*) serum, anti-Bedford's red-backed vole (*Clethrionomys rufocanus bedfordiae*) serum, anti-bovine (*Bos taurus domesticus*) serum, anti-horse (*Equus caballus*) serum, anti-sheep (*Ovis aries*) serum, anti-human serum and anti-chicken (*Gallus gallus domestica*) serum. The former two sera were prepared in the laboratory using New Zealand white rabbits by the procedure described earlier (14). The anti-Bedford's red-backed vole serum produced in rabbits was obtained from Dr. K. Takahashi of the Hokkaido Institute of Public Health. The other sera were commercial sera produced in rabbits (Miles-Yeda Ltd.).

The methodology for checking prepared antisera and the results of the check were described earlier (14).

## 3. Conjugate and Substrate

Commercial horse radish peroxidase conjugated to the IgG fraction of goat anti-rabbit serum (Miles-Yeda Ltd.) and *o*-phenylene diamine dissolved in citric acid- $\text{Na}_2\text{HPO}_4$  and  $\text{H}_2\text{O}_2$  were used as described earlier (14).

## 4. ELISA procedure

The methodology as adapted for this study was described earlier (14), except for the following modifications: 1% gelatin dissolved in phosphate buffered saline (PBS), pH 7.2 was used for the coating buffer.

## Results and Discussion

A total of 813 individuals out of 18 649 (4.36%) in *P. jezonicum* (Table 1) and 120 out of 206 (58.25%) in *P. yezoense* were observed to have blood-meals in their alimentary canal in this investigation (Table 2).

The blood-meals of 108 individuals of *P. jezonicum* reacted against the anti-sera of 5 mammal species namely human, cattle, horse, Bedford's red backed vole and sika deer (Table 3). Of these, Bedford's red backed vole and sika deer are determined as the blood source animals of *P. jezonicum* for the first time. The other species, human, cattle and horse were reported to be the blood sources of *P. jezonicum* (7), but the report is based on the results of human and animal attractant methods, therefore, some doubt

remained to regard those three mammal species to be the blood source animals of *P. jezonicum*. And moreover, this species had been confused with *P. hiltipes* for a long time (10), (17), thus the species which Ogata reported cannot be specified to be *P. jezonicum* (9).

The blood-meals of 47 individuals of *P. yezoense* reacted against anti-bovine, -horse, -human, -sheep and -Bedford's red backed vole sera (Table 4).

**Table 1.** Number of *P. jezonicum* collected in this investigation

locality	date	collected No.	engorged No.	(%)
Mt. Onnebetsu	1984. 8. 1	12	8	66.67
Nukabira	1984. 5. 18	248	245	98.79
Hobetsu	1984. 5. 20	19	7	2.70
Tohbetsu	1985. 5. 23	11223	303	13.33
Nopporo	1985. 6. 4	15	2	13.33
Tohbetsu	1985. 6. 6	5934	228	3.84
	1985. 6. 20	1187	15	1.26
	1985. 7. 4	11	5	45.45
Total		18649	813	4.36

**Table 2.** Number of *P. yezoense* collected in this investigation

locality	date	collected No.	engorged No.	(%)
Mt. Onnebetsu	1984. 8. 1	2	1	50.00
Tohbetsu	1985. 6. 6	2	1	50.00
	1985. 6. 20	9	4	44.44
	1985. 7. 4	59	17	28.81
Johzankei	1985. 7. 17	7	3	42.86
Hitsujiogaoka	1986. 6. 8	1	1	100.00
	1986. 6. 24	10	10	100.00
Okushiri Isl.	1986. 6. 27	7	5	71.43
	1986. 6. 28	46	40	86.96
Hitsujiogaoka	1986. 7. 8	8	8	100.00
Tohbetsu	1986. 7. 17	11	2	18.18
Okushiri Isl.	1986. 7. 18	30	24	80.00
	1986. 7. 19	2	0	0.00
Shibechea	1986. 7. 26	1	0	0.00
Mt. Oohirayama	1986. 8. 1	4	2	50.00
	1986. 8. 3	7	2	28.57
Total		246	120	58.25

**Table 3.** Number and ratio (%) of *P. jezoanicum* reacted their blood-meals with anti-human and animal sera

locality	date	examined number	number and percent of black flies reacted against									
			anti-bovine	anti-horse	anti-sheep	anti-human	anti-voile	anti-sika deer	anti-brown bear	anti-birds		
Mt. Onnebetsu	1984. 8. 1	8	0 (0.0)	0 (0.0)	0 (0.0)	2 (25.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Nukabira	1984. 5. 18	245	0 (0.0)	0 (0.0)	0 (0.0)	4 (1.6)	2 (0.8)	10 (4.1)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Hobetsu	1984. 5. 20	7	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Tohbetsu	1985. 5. 23	303	19 (6.3)	21 (6.9)	0 (0.0)	17 (5.6)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Nopporo	1985. 6. 4	2	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Tohbetsu	1985. 6. 6	228	19 (8.3)	6 (2.6)	0 (0.0)	3 (1.3)	4 (1.8)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
	1985. 6. 20	15	0 (0.0)	1 (6.7)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
	9185. 7. 4	5	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Total		813	38 (4.7)	28 (3.4)	0 (0.0)	26 (3.2)	6 (0.7)	10 (1.2)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)

**Table 4.** Number and ratio (%) of *P. yezoense* reacted their blood-meals with anti-human and animal sera

locality	date	examined number	number and percent of black flies reacted against									
			anti-bovine	anti-horse	anti-sheep	anti-human	anti-voile	anti-sika deer	anti-brown bear	anti-birds		
Mt. Onnebetsu	1984. 8. 1	1	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Tohbetsu	1985. 6. 6	1	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
	1985. 6. 20	4	2 (50.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
	1985. 7. 4	17	1 (5.9)	1 (5.9)	0 (0.0)	0 (0.0)	2 (11.8)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Johzankei	1985. 7. 17	3	1 (33.3)	0 (0.0)	0 (0.0)	1 (33.3)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Hitsujiogaoka	1986. 6. 8	1	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
	1986. 6. 24	10	1 (10.0)	1 (10.0)	1 (10.0)	3 (30.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Okushiri Isl.	1986. 6. 27	5	1 (20.0)	3 (60.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
	1986. 6. 28	40	3 (7.5)	8 (20.0)	0 (0.0)	10 (25.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Hitsujiogaoka	1986. 7. 8	1	1 (12.5)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Tohbetsu	1986. 7. 17	2	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Okushiri Isl.	1986. 7. 18	24	0 (0.0)	2 (8.3)	0 (0.0)	5 (20.8)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Mt. Oohirayama	1986. 8. 1	2	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
	1986. 8. 3	2	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Total		120	10 (8.3)	15 (12.5)	1 (0.8)	19 (15.8)	2 (3.9)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)

Of these mammal species, sheep and Bedford's red backed vole are determined as the blood source animals of *P. yezoense* for the first time. Ogata (1955) and Ono (1980) reported that human, cattle and horse to be the blood source animals of this species. And the results of this study confirmed their report immuno-serologically.

The two species examined in this study have simple shaped tarsal claws but with small sub-basal tooth, and the authors included the species into S-type group 13). The black flies having S-type tarsal claws are considered to be mammalphylic blood suckers 1), 15) and none of the blood-meal examined reacted against anti-birds serum in this study. Therefore, the results obtained in this study are solid supports to the conclusion.

### Summary

The blood-meals of two major *Prosimulium* species of Hokkaido, Japan were examined immuno-serologically by means of enzyme-linked immunosorbent assay (ELISA) in order to determine the blood source animals of the black flies. A total of 108 indiv. out of 18 649 in *P. jezonicum* and 47 indiv. out of 206 in *P. yezoense* were determined their blood-meals.

The blood-meals of *P. jezonicum* reacted against anti-human, -bovine, -horse, -Bedford's red backed vole and -sika deer sera and those of *P. yezoense* reacted against anto-bovine, -horse, -human, -sheep and -Bedford's red backed vole sera. None of the blood-meals reacted against anti-birds serum.

Bedford's red backed vole and sika deer are determined to be the blood source animals of *P. jezonicum* and sheep and Bedford's red backed vole are determined to be the blood source animals of *P. yezoense* for the first time.

The two black fly species have S-type tarsal claws and black flies having S-type tarsal claws are considered to be mammalophilic blood suckers. The results obtained in this study provide solid support of this conclusion.

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## 要 約

吸血源動物を知る目的で、北海道に分布する *Prosimulium* 属の主要種であるオオブユとキアシオオブユの2種の blood-meal を酵素抗体法を用いて、免疫学的に同定した。

その結果、オオブユでは抗ヒト、抗ウシ、抗ウマ、抗エゾヤチネズミ、抗エゾシカ血清に、キアシオオブユでは抗ヒト、抗ウシ、抗ウマ、抗ヒツジ、抗エゾヤチネズミ血清にそれぞれ陽性反応を示したが、抗鳥類血清に陽性反応を示した個体はなかった。このうち、オオブユにおけるエゾヤチネズミとエゾシカ、キアキオオブユにおけるヒツジとエゾヤチネズミはそれぞれ吸血源動物として本報においてはじめて確認された。

また、これら2種のブユの跗節の爪は歯を持たない S-type で、ほ乳類吸血性と見られていたが、本報に示された結果からこのことが確認された。