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## Crossing Experiments among Three Ladybird "Species" of *Henosepilachna vigintioctomaculata* Complex (Coleoptera, Coccinellidae) Feeding on Thistles and/or Blue Cohosh\*

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**Synopsis** Among three closely related "species" in group B of *Henosepilachna vigintioctomaculata* complex, *H. niponica* and *H. yasutomii* are sympatric and respectively feed on thistles and blue cohosh, while *H. pustulosa* is allopatric with the other "species" and feeds on thistles or both thistles and blue cohosh. Under laboratory conditions, these three "species" easily interbreed with each other and produce F<sub>1</sub> hybrids as viable and fertile as respective homogamic offspring, showing their close genetic affinity and very incomplete post-mating reproductive isolation. This suggests the presence of a strong pre-mating isolation between two sympatric "species", *H. niponica* and *H. yasutomii*. But such a strong pre-mating isolation is ecologically difficult to assume between allopatric "species", i.e., *H. pustulosa* vs. *H. niponica* and *H. yasutomii*, due to the duplication of their host plants. Thus, it is likely that these "species" as a whole are still on the borderline of good biological species, nevertheless *H. niponica* and *H. yasutomii* have been sufficiently isolated with each other under sympatric natural conditions.

A series of closely related phytophagous ladybirds in Japan belonging to *Henosepilachna vigintioctomaculata* complex has been paid much attention from the viewpoint of speciation. In a previous paper, KATAKURA (1981) divided this complex into two groups, A and B. Group A is composed of a single species, *H. vigintioctomaculata* (MOTSCHULSKY), which is a notorious pest of potato (*Solanum tuberosum* L., Solanaceae) in the cool temperate northeastern Asia. On the other hand, group B includes very diverse forms varying in external morphology and food habits. All members of group B are known only from Japan and in most cases dependent on wild plants other than Solanaceae. Based on the morphological evidence and geographic sympatric-allopatric relations, group B was further divided into three subgroups, which were tentatively treated as three separate species, *H. niponica* (LEWIS), *H. pustulosa* (KÔNO) and *H. yasutomii* KATAKURA. Among these three "species", *H. niponica* and *H. yasutomii* are widely sympatric with each other in the southern area of the distribution range of group B (Honshu and the southernmost part of Hokkaido, Fig. 1), feeding on different host plants, thistles (*Cirsium* spp., Compositae) and blue cohosh (*Caulophyllum robustum* MAXIM., Berberidaceae), respectively. On the other hand, *H. pustulosa* is allopatric with the

\* Contributions to the knowledge of *Henosepilachna vigintioctomaculata* complex. XI.

former two "species", occupying the northern area (Hokkaido except for the southernmost part, Fig. 1) and mainly depending on thistles (forms P-III', I, II) or both thistles and blue cohosh (P-III). *H. niponica* and *H. yasutomii* are easily separable from each other by the elytral shape and body size, but the separation of *H. pustulosa* from these two "species" by the same characters is virtually impossible. *H. pustulosa* has a body size intermediate between *H. niponica* and *H. yasutomii*. Further, the elytral shape can not be used as the diagnostic character for allopatric populations due to the conspicuous geographic variation in both *H. niponica* and *H. pustulosa*. Thus, group B is represented by two "species" in the southern area while by a single "species" in the northern area, but the relation between one northern and two southern "species" is not clearly understood yet.

The present paper is an outcome of crossing experiments among these three "species" made in 1980 to get further information on the relation between one northern and two southern "species" and to know the degree and extent of post-mating isolation between sympatric "species", *H. niponica* and *H. yasutomii*.

Before going further we wish to express our heartiest thanks to Prof. Shōichi F. SAKAGAMI, Hokkaido University, for his deep interest to the present study and critical reading of the manuscript. We are indebted to Messrs. Kazuo HOSHIKAWA and Susumu NAKANO who allowed us to cite their unpublished data. This study was partly supported by a grant from the Ministry of Education (No. 574303) to one of us (HK).

### Materials and Methods

Henceforth the three "species" are abbreviated as follows: *H. pustulosa*, Hp or P; *H. niponica*, Hn or N; *H. yasutomii*, Hy or Y. The materials were chosen among the southernmost member of Hp (form P-III, a thistle and blue cohosh feeder) and the northernmost members of Hn (form N-I, a thistle feeder) and Hy (a blue cohosh feeder) (Fig. 1). Larvae and prepupae of each form collected at the field were reared with the host plant on which they were caught (Table 1). To synchronize the emergence, immatures of Hy were reared under a lower temperature than those of Hn and Hp. Soon after the emergence, females and males of each form were separated and reared with potato leaves for approximately two weeks to assure the ovarian maturation. Crossings were made in a transparent plastic case. The pair whose mating lasting more than 60 min without marked rejection by the female (cf. KATAKURA & NAKANO, 1979) was isolated and onwards kept together in a transparent plastic case with fresh potato leaves. A total of nine combinations, three homogamic and six heterogamic ones, was prepared. Eggs laid by each pair were counted every day and their hatching ratio was examined. Some larvae hatched from these eggs were reared with a herb, *Solanum megacarpum* KOIDZ., which was collected every two or three days at Nopporo and stored in the laboratory. The lots producing both sexes of offspring adults were selected and successively reared

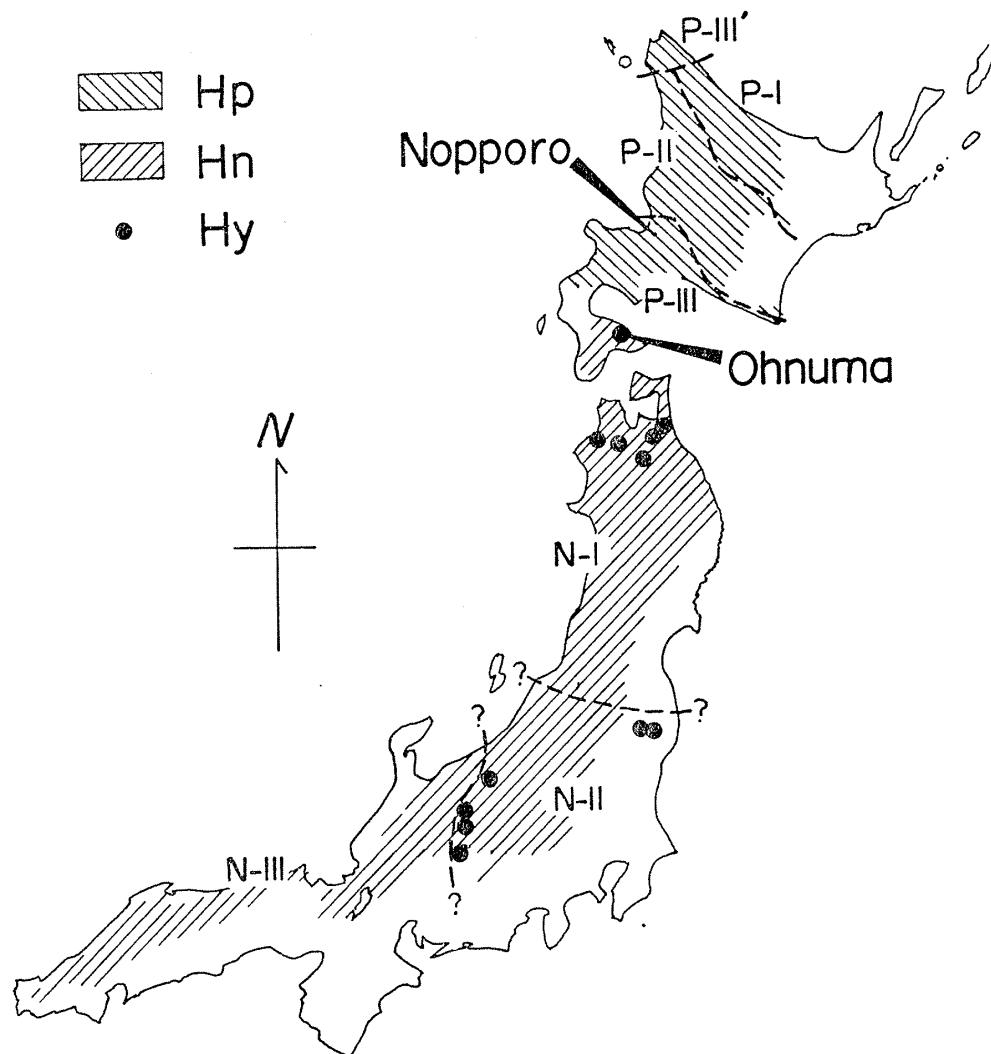


Fig. 1. Geographic distribution of *H. pustulosa* (P-III', P-I~III), *H. niponica* (N-I~III) and *H. yasutomii*, showing the provenance of materials used. Distribution ranges of *H. pustulosa* and *H. niponica* are greatly simplified. (Modified from KATAKURA, 1981)

Table 1. Source of materials and rearing conditions of their immature stages.

Species (form)	at	Collected		Stages collected*	Rearing conditions	
		on	from		Environment	Food
Hp (P-III)	Nopporo	vii, 21	thistles	3L to PP	24°C, 16L8D	thistles
Hn (N-I)	Ohnuma	vii, 9	ditto	3L to 4L	ditto	ditto
Hy	ditto	ditto	blue cohosh	3L to PP	20°C, 16L8D	blue cohosh

\* 3L, 4L: third and fourth instar larvae; PP: prepupae.

with potato or *S. megacarpum*. The hatching ratio of their eggs in each lot was examined to know the F<sub>1</sub> fertility. After the emergence of the parent insects, all rearings were made under a constant temperature (24°C) and daylength (16L8D).

As mentioned above, the plants offered during experiments were potato and *S. megacarpum*, which were different from those usually fed by the ladybirds. Normal growth of Hp (P-III) by these plants was already confirmed (HINOMIZU, 1976; NAKANO, unpubl.) but viability of other two "species" by these plants was not yet exactly known. Therefore, the food condition might have affected the results given below.

### Results

1. *Hatching ratio.* Totally 21 homogamic and 57 heterogamic pairs were examined (Tables 2 and 3). Number of pairs examined for each combination varied from four to eleven. Besides the pairs given in these tables, two pairs of Y ♀ × Y ♂, two of P ♀ × Y ♂ and three of Y ♀ × P ♂ were made by using adults reared from eggs under uncontrolled room condition with potato leaves (collected in mid June from the same populations of respective "species" given in Table 1). However, the hatching ratios of eggs laid by these pairs were considerably low: Y ♀ × Y ♂, 1.1, 2.4%; P ♀ × Y ♂, 22.4, 52.2%; and Y ♀ × P ♂, 5.1, 1.4, 2.7%. Since the low hatchability in the homogamic pairs suggests aberrant physiological condition of the adults involved in these pairs, the results concerning them were omitted from the considerations.

*Homogamic combinations (Table 2):* The hatching ratios were different among three homogamic combinations, being 4.3–52.3% (mean 34.1%) in Hn, 46.5–83.8% (65.5%) in Hp, and 73.7–82.4% (77.3%) in Hy. The values here obtained for Hn and Hp, especially the former, were lower than those for *H. vigintioctomaculata* (mean 71.6%) and *H. pustulosa* (74.2%) previously reported by KATAKURA and NAKANO (1979). Different responses of these forms to the rearing conditions (food and/or environment) may have resulted in the difference of their hatching ratios.

*Heterogamic combinations (Table 3):* Although the hatching ratios fluctuated

Table 2. Hatching ratios of eggs laid by homogamic pairs.

Combination (mean±SD in %)	Pair code									Total	
	1	2	3	4	5	6	7	8	9		
N♀ × N♂ (34.1±17.3)	a*	275	309	184	213	457	203	362	149		2152
	b*	92	132	8	43	239	44	165	78		801
	c*	33.5	42.7	4.3	20.2	52.3	21.7	45.6	52.3		37.2
P♀ × P♂ (65.5±13.9)	a	202	232	475	179	283	421	86	299	195	2372
	b	94	160	227	94	220	353	59	238	124	1569
	c	46.5	69.0	47.8	52.5	77.7	83.8	68.6	79.6	63.6	66.1
Y♀ × Y♂ (77.3±3.8)	a	84	297	237	245						863
	b	65	219	179	202						665
	c	77.4	73.7	75.5	82.4						77.1

\* a: number of eggs laid; b: ditto, hatched; c: percentage hatched.

Table 3. Hatching ratios of eggs laid by heterogamic pairs.

Combination (mean $\pm$ SD in %)	Pair code											Total
	1	2	3	4	5	6	7	8	9	10	11	
N♀ × P♂ (30.4 $\pm$ 24.7)	a*	252	217	45	396	160	214	197	528	409	278	2696
	b*	146	126	28	0	75	12	59	85	1	76	608
	c*	57.9	58.1	62.0	0	46.9	5.6	29.9	16.1	0.2	27.3	22.6
P♀ × N♂ (65.1 $\pm$ 14.3)	a	117	231	20	382	452	234	105	206	132	75	1854
	b	81	133	17	271	331	119	38	157	77	55	1279
	c	69.2	57.6	85.0	70.9	73.2	50.9	36.2	76.2	58.3	73.3	69.0
N♀ × Y♂ (34.1 $\pm$ 21.8)	a	277	57	238	425	306	228	318	48	231	285	2413
	b	3	31	85	176	136	177	79	13	50	38	788
	c	1.1	54.4	35.7	41.4	44.4	77.6	24.8	27.1	21.6	13.3	32.7
Y♀ × N♂ (34.6 $\pm$ 27.3)	a	63	149	41	33	270	194	256	108	48	67	1338
	b	50	57	0	0	119	103	80	13	2	39	528
	c	79.4	38.3	0	0	44.1	53.1	31.3	12.0	4.2	59.6	39.5
P♀ × Y♂ (57.8 $\pm$ 22.2)	a	226	179	410	204	36	153	327	261			1796
	b	113	112	335	153	18	21	257	132			1141
	c	50.0	62.6	81.7	75.0	50.0	13.7	78.6	50.6			63.5
Y♀ × P♂ (72.0 $\pm$ 14.5)	a	189	201	185	192	390	238	145	239			1779
	b	134	74	149	152	304	179	111	188			1291
	c	70.9	36.8	80.5	79.2	77.9	75.2	76.6	78.7			72.6

\* a: number of eggs laid; b: ditto, hatched; c: percentage hatched.

Table 4. Mean hatching ratios of eggs laid by various combinations of Hn, Hp and Hy. Those of homogamic combinations are given by Gothic.

Male	Female		
	Hn	Hp	Hy
Hn	<b>34.1</b>	65.1	34.6
Hp	30.4	<b>65.5</b>	72.0
Hy	34.1	57.8	<b>77.3</b>

Table 5. Larval period of F<sub>1</sub> offspring in various crossings among Hn, Hp and Hy. (24°C, 16L8D, reared with *S. megacarpum*).

Combination	Number of F <sub>1</sub> reared to adult (♀♀/♂♂)	Larval period (days)						
		mean	17	18	19	20	21	<22
<b>Homogamic</b>								
N ♀ × N ♂	10 (5/5)	20.4	0	0	3	3	1	3
P ♀ × P ♂	15 (4/11)	18.7	0	5	10	0	0	0
Y ♀ × Y ♂	25 (12/13)	18.4	5	10	4	6	0	0
<b>Heterogamic</b>								
N ♀ × P ♂	21 (9/12)	19.0	0	4	14	2	1	0
P ♀ × N ♂	28 (12/16)	19.5	0	5	6	14	3	0
N ♀ × Y ♂	32 (15/17)	18.8	2	9	17	3	1	0
Y ♀ × N ♂	30 (15/15)	19.5	0	4	17	6	0	3
P ♀ × Y ♂	44 (28/16)	18.2	4	27	13	0	0	0
Y ♀ × P ♂	31 (18/13)	18.1	0	28	3	0	0	0

within and among combinations, they are approximately included within the range of homogamic combinations. The mean and range of the ratios in each heterogamic combination are as follows: N ♀ × P ♂, 30.4% (0–62.2%); P ♀ × N ♂, 65.1% (36.2–85.0%); N ♀ × Y ♂, 34.1% (1.1–77.6%); Y ♀ × N ♂, 34.6% (0–79.4%); P ♀ × Y ♂, 57.8% (13.7–81.7%); and Y ♀ × P ♂, 72.0% (36.8–80.5%).

Table 4 synoptically shows the mean hatching ratios of nine combinations in the present study. The hatching ratio of eggs laid by females of each “species” is fairly constant irrespective of the species of their partners. The ratios become higher in the order of Hn ♀ < Hp ♀ < Hy ♀. The exception from this trend is given by the combination Y ♀ × N ♂, where the hatching ratio was considerably low seen from such a point of view. The high hatching ratios of eggs laid by various heterogamic combinations among Hp, Hn and Hy are quite dissimilar to those by heterogamic combinations between *H. pustulosa* and *H. vigintioctomaculata*, in which only a few percent of eggs hatched (KATAKURA & NAKANO, 1979).

2. *Growth.* In total 186 hybrid adults together with 50 homogamic offspring ones were obtained. Since the immatures were reared under somewhat unfavorable conditions, many offspring individuals suffered from an unknown disease. Therefore, the larval mortality could not be examined in the present study. Instead, the

Table 6.  $F_1$  fertility shown by the hatching ratios of eggs laid by various types of  $F_1$  offspring among Hn, Hp and Hy. Type of  $F_1$  is given by the combination of the parents. For each type data of more than one lot were combined and each lot contained one to five females.

Type of $F_1$ offspring	No. of lots combined	No. of adults involved ( $\text{♀♀}/\text{♂♂}$ ) beginning end		Total no. of eggs laid hatched		Percentage hatched Total (Each lot)
Homogamic						
$N\text{♀}N\text{♂}$	3	4/4	3/3	513	249	48.5 (50.6, 54.7, 39.2)
$P\text{♀}P\text{♂}$	1	4/2	4/1	238	160	67.2
$Y\text{♀}Y\text{♂}$	3	7/8	7/7	537	163	30.4 (44.4, 28.2, 30.4)
Heterogamic						
$N\text{♀}P\text{♂}$	3	9/9	9/6	895	252	28.2 (30.8, 35.2, 0)
$P\text{♀}N\text{♂}$	1	4/11	3/10	20	13	65.0
$N\text{♀}Y\text{♂}$	5	7/10	7/10	680	220	32.4 (8.0, 50.5, 17.5, 42.9, 51.9)
$Y\text{♀}N\text{♂}$	3	7/8	7/6	623	114	18.3 (10.3, 30.6, 28.2)
$P\text{♀}Y\text{♂}$	4	10/11	7/9	574	276	48.1 (35.4, 72.9, 48.7, 20.0)
$Y\text{♀}P\text{♂}$	3	8/8	7/5	384	173	45.1 (59.4, 27.0, 26.2)

larval period was calculated for the individuals growing to the adult stage (Table 5). Although the number of individuals examined for each combination was insufficient, the mean larval periods of three homogamic combinations were 20.4 (Hn), 18.7 (Hp) and 18.4 days (Hy), and of heterogamic ones 18.1–19.5 days. No marked delay or acceleration was observed in the larval periods of all types of  $F_1$  hybrids examined. In the crossings  $Hp \times Hn$  and  $Hn \times Hy$ , the larval periods of the hybrids were intermediate between those of the parent "species", while in  $Hp \times Hy$ , the larval period was similar to that of Hy parent. In all types of offspring adults obtained by the rearing, the sex ratio did not significantly biased from 1:1 ( $P > 0.05$ ).

3. *Fertility of hybrids.* By successive rearing of some lots of offspring adults with both sexes, we obtained eggs from all types of sib matings of offspring. Table 6 shows the hatching ratios of these eggs. The hatching ratio was not examined for each female separately. Further, these eggs may have involved unfertilized ones since mating was not confirmed in this case. Therefore, accurate comparison of the ratios given in the table was difficult. However, the hatching ratios of eggs laid by all types of  $F_1$  hybrids were not so lower than those of homogamic ones, suggesting a normal fertility of both sexes of hybrids among Hp, Hn and Hy.

### Discussion

As mentioned above, we could not find any abnormal trait in the hatchability of eggs and viability and fertility of  $F_1$  hybrids produced by the interspecific crossings among Hp, Hn and Hy, except for  $Hy \text{♀} \times Hn \text{♂}$ , where the hatchability seemed somewhat lower than those in all other combinations examined. This means a very close genetic affinity of these three "species". From the viewpoint of reproduc-

tive isolation, the present study shows a very incomplete post-mating isolation among these "species", though this does not preclude the possibility of hybrid breakdown due to unknown deleterious traits of their hybrids. As to the sympatric "species" Hn and Hy, therefore, we can predict the presence of a strong pre-mating isolation between them. Hn depends on thistles, while Hy on blue cohosh. Even under laboratory conditions, both adults and larvae of Hy virtually refused thistles and those of Hn had a very low preference for blue cohosh (KATAKURA, 1976; HOSHIKAWA, pers. comm.). This host plant difference is apparently an important isolating factor. Another possible factor which needs further investigation may be the sexual isolation between them.

On the other hand, Hp is allopatric with both Hn and Hy. Comparing some characteristics of Hp with Hn and Hy, KATAKURA (1981) enumerated the following four alternative interpretations about the relation of these three forms, but without particular emphasis on any of them:

Case 1: Three distinct species, Hp, Hn and Hy are involved.

Case 2: Hy and Hp are conspecific, Hn is another species.

Case 3: Hn and Hp are conspecific, Hy is a separate species.

Case 4: Three forms lie on the borderline of good species, and likely in the process of speciation; Hn and Hy completed the reproductive isolation between them but neither is yet fully isolated from Hp.

All these cases are still possible but cases 1-3 are less probable when the present results are taken into account. These cases require the potential strong pre-mating isolation between Hp and either Hn (case 2) or Hy (case 3) or the both (case 1) as was supposed between Hn and Hy. However, it is difficult to imagine such a strong isolation within the limit of our present knowledge. Hp (strictly speaking P-III) actually utilizes both thistles (the host plant of Hn) and blue cohosh (that of Hy) under natural conditions. Therefore, at least the difference of the host plant, which is considered as one of the important pre-mating isolating factors between Hn and Hy, is not effective in these cases. Consequently case 4 is most likely. It is conceivable that, when Hp becomes sympatric with Hn and/or Hy, they may be intermingled on the same kind of host plant and at least partly hybridize with each other. Apparently the above assumption is still inconclusive. Further comparative studies on the life history, food habits and nature of hybrids of these "species" will clarify their relations in more detail.

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### 新刊紹介

**Manual of Nearctic Diptera.** Vol. 1. Coordinated by J. F. McALPINE, B. V. PETERSON, G. E. SHEWELL, H. J. TESKEY, J. R. VOCKEROTH and D. M. WOOD. Research Branch Agriculture Canada Monograph No. 27. vi+674 pp., 2027 figs. Minister of Supply and Service Canada. 1981.

世界の双翅類研究者の待望久しい本書は、カナダの Biosystematics Research Institute の双翅類研究者が中心になり、世界の権威者 50 名が執筆陣に加わり、故 CHILLCOTT と HENNIG の協力を得て完成されたものである。本書は基本的には新北区の双翅類の成虫の属を検索するためのものであるが、わが国でも双翅類研究者の良き指針であった C. H. CURRAN の旧著 *Families and Genera of North American Diptera* を一新した内容である。第 1 巻は序、成虫と幼虫の形態、成虫と幼虫の科の検索表に続いて、長角群のニセヒメガガンボ科から短角群のアシナガバエ科までが扱われている。残りの全ての科を扱う第 2 巻も近く出版される予定という。序に続く第 2 章の成虫の形態を McALPINE が分担し、♂ 交尾器を含む外部形態を詳述しており、その内容は単に分類に用いる形態形質の名称の説明にとどまらない。従来、分類群によって名称の不統一や混乱が見られた♂ 交尾器についても、彼自身の研究に基づいた統一的解釈がなされている。幼虫の形態は TESKEY が分担し、頭部の形態や気管系など、分類上重要な形質を解説している。第 4 章は新北区産の双翅目の全 108 科の成虫の科の検索表、第 5 章は 91 科の幼虫の科の検索表である。第 6 章以後は 1 章 1 科だてで構成され、各科ごとに成虫の形態学的特徴が詳述され、幼虫や蛹の形態、生態や行動、分類と分布についても世界的なレベルでの適切なレビューが示されている。新北区産属（必要に応じて亜属）の成虫の検索表は多数の付図があって大変使いやすく、属ごとに北米産の種数と分布の概要が示されている。さらにガガンボ、カ、ブユ、アブなど多くの科については幼虫や蛹の属の検索表も掲げられている。各章末にはその科の分類、幼虫期、形態などに関する主要文献が列挙されている。巻末には分類群名と形態学用語の索引が付されていて便利である。全巻にわたって挿入された夥しい数の極めて精緻で統一的な形態図が、本書の価値を著しく高めている。各科ごとに主に模式属の成虫の全形図が示されているのも、そのハエの概形を知る上で参考になる。双翅目は一般にはたいへんとりつきにくい群であり、わが国に分類関係の良書もない現状では、本書は双翅目の概要を知る上で唯一のしかも最新の内容を盛りこんだものとして必携のものであり、新北区と関連の深いわが国の双翅類の属の所属を知る上でも大いに活用できよう。双翅類の分類学関係者のみならず、広く昆虫分類学者、生態学者、農学や医学、環境科学関係の応用昆虫学者や研究機関、さらにアマチュア研究者にも推薦したい。価格も 48 カナダドルで廉価である。入手先は次の通り： Canadian Government Publishing Centre, Supply and Services Canada, Hull, Quebec, Canada KIA OS9.

(三枝 豊平)