IS THE HORAANA STRAIN OF THE *CULEX PIPIENS* GROUP *CULEX PIPIENS PALLENS*?

TAKASHI ISHII AND SOHN SUCK RAK[1]

College of General Education, University of Tokushima, 1-1 Minamijosanjima, Tokushima 770 Japan

ABSTRACT. The validity of the Horaana strain of the *Culex pipiens* group as *Cx. pipiens pallens* was studied. Judging from the fourth instar characters and morphology of the adult male genitalia, this laboratory strain is now out of the range of ordinary *Cx. p. pallens* in Japan, and must be identified as *Cx. p. quinquefasciatus.*

INTRODUCTION

The Horaana strain was originally collected in January 1964 by K. Saito as hibernating adults from a cave (*horaana* in Japanese) in Kawasaki (35°30′N, 139°45′E) in the suburbs of Tokyo. It has been maintained in many prominent institutions in Japan and used in experiments as "the standard *pallens*-form from the Tokyo area" [Sasa et al. 1966; whose work is widely referred to in papers concerned with the *Cx. pipiens* group, such as that of Barr (1986)]. However, careful examination of fourth-instar characters such as the shape of the siphon, the siphonal index and the numbers of branches of the first siphonal setal tuft has revealed that this strain is morphologically beyond the normal range of *Cx. p. pallens* Coquillett from central Japan, but close to *Cx. p. quinquefasciatus* Say (Ishii 1983a, 1983b). Subsequent investigations of the adult male genitalia have confirmed this.

MATERIALS AND METHODS

Adult males as well as larvae from the Horaana strain studied earlier (Ishii 1983a) were obtained from three laboratories which had maintained it independently for a long time: the F_{167} generation (TKU) from Dr. T. Kurihara of the Medical School of Teikyo University (received on May 22, 1981), the F_{173} (TWMC) from Dr. Y. Wada of the Department of Parasitology, Tokyo Women's Medical College (July 5, 1982), and the F_{176} (IMS) from Dr. A. Shirasaka of the Department of Parasitology, Institute of Medical Sciences, University of Tokyo (July 2, 1982), which has cultured this strain continuously from its establishment.

For comparison, we examined male *Cx. p. pallens* (YCH) collected by Dr. M. Ikeuchi on June 22, 1981 in Yachiyo (35°46′N, 140°06′E) near the collection site of the Horaana strain and F_{119} generation (OGS) of *Cx. p. quinquefasciatus* originally collected in Ogasawara (27°06′N, 142°10′E), maintained in the Institute of Medical Sciences, and sent by Dr. A. Shirasaka (received July 2, 1982).

The male genitalia were mounted in Canada balsam by the usual methods. Measurements were made of V (the distance between the points of the ventral arms) and D (the distance between the tips of the dorsal arms) by a direct-reading micrometer for calculation of the DV/D ratio [= (V − D)/2D] (Fig. 1).

RESULTS

Male genitalia of the Horaana strain resemble those of OGS but are different from those of YCH (Fig. 1).

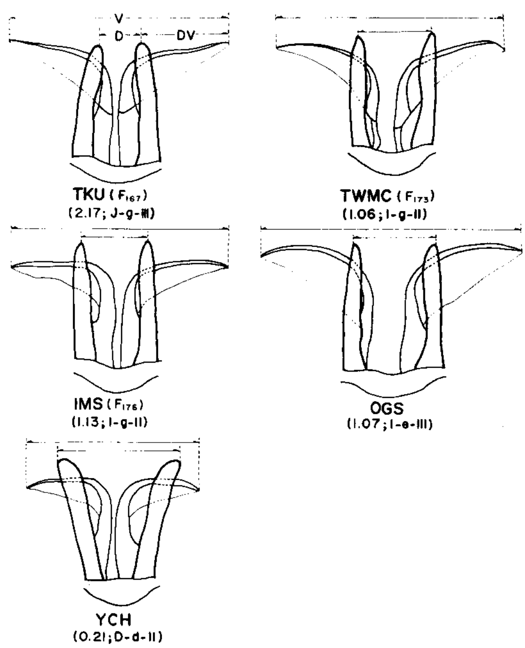

Fig. 1. Some male genitalia of the three Horaana colonies, OGS and YCH. DV/D and combinations of the DA-, VA-, and DVP-types are in parentheses.

[1] Present address: Biology Department, Taegu National Teacher's College, 1797-5, Daemyeong-2dong, Namgu, Taegu 634, Korea.

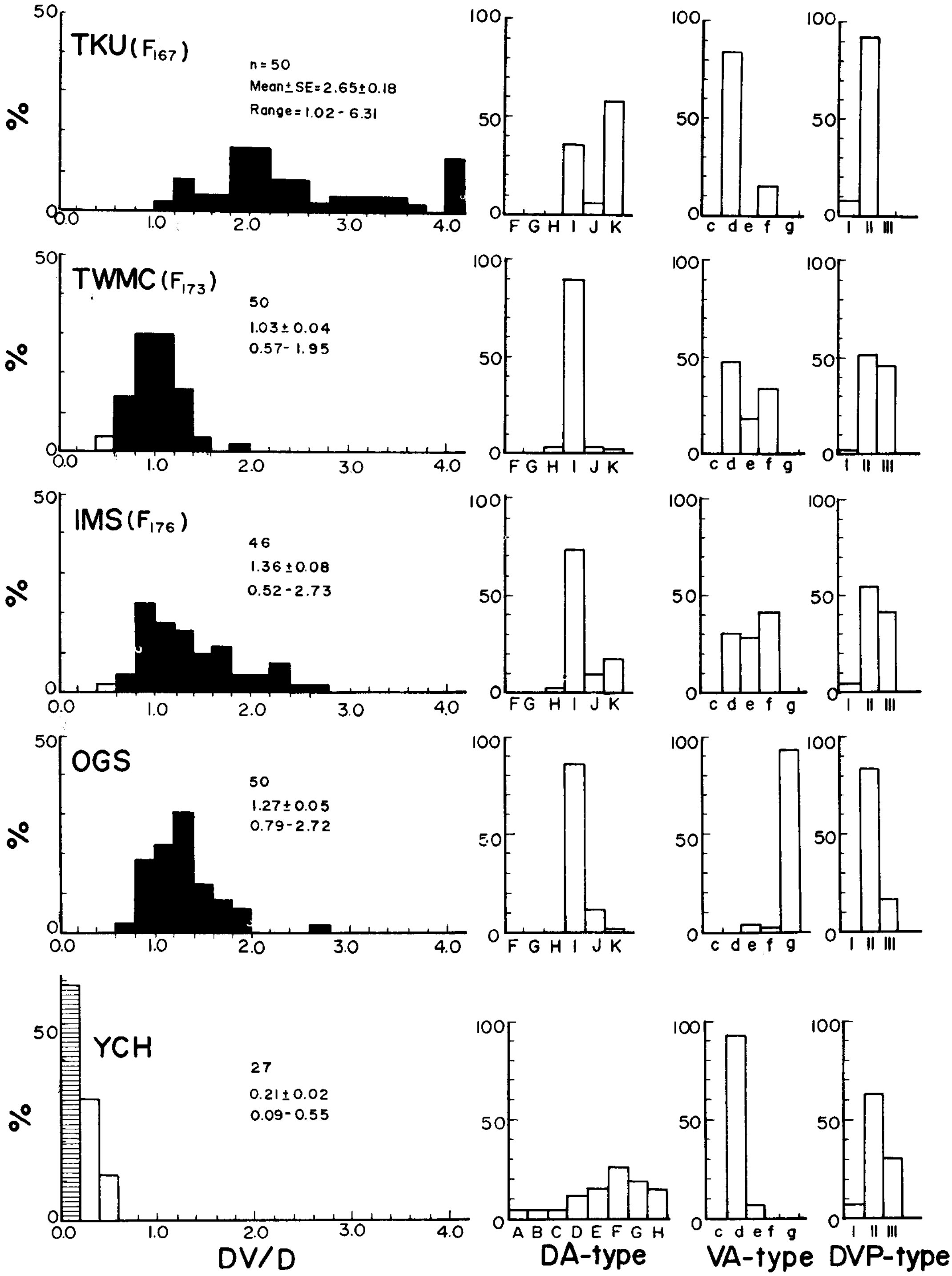

Fig. 2. Frequency distribution of the DV/D, DA-, VA-, and DVP-types in the three Horaana colonies, OGS and YCH.

We recognize 13 types (types A to K, Z_1 and Z_2) of the dorsal and seven (types a to g) of the ventral arms (the DA-type and VA-type), respectively, and 3 types (types I to III) in the relative position of the two arms (the DVP-type), in *Cx. p. pipiens* Linn., *Cx. p. pallens*, *Cx. p. quinquefasciatus* and *Cx. p. molestus* Förskal from material collected worldwide. Types A to

Table 1. The significance level of the differences in mean DV/D by the Cochran-Cox method and that in the distribution patterns of the DA-, VA- and DVP-types in the Chi-square test.

Sample	n	Mean DV/D ± SE*	DA-type	VA-type	DVP-type
TKU (F_{167})	50	2.65 ± 0.18 c	b	b	a
TWMC (F_{173})	50	1.03 ± 0.04 a	a	a	ad
IMS (F_{176})	46	1.36 ± 0.08 ab	c	a	ad
OGS	50	1.27 ± 0.05 b	a	c	c
YCH	27	0.21 ± 0.02 d	d	d	d

* Mean followed by the same letter and distribution patterns indicated by the same letter within a column do not differ significantly ($P < 0.05$).

C and a and b are common in *Cx. p. pipiens*, types D to H and c and d are common in *Cx. p. pallens* and types I to K and e to g are common in *Cx. p. quinquefasciatus*. All three DVP-types can be recognized in the subspecies mentioned above (Sohn 1987).[2] In the three Horaana colonies, 4 types (I to K in TKU; H to K in TWMC and IMS) were recognized in the DA-type, 3 (d and f in TKU; d to f in TWMC and IMS) in the VA-type, and 3 in the DVP-type, while types I to K and d to f in OGS, and A to H and d and e in YCH.

Figure 2 illustrates the frequency distributions of the DV/D, DA-, VA-, and DVP-types in the three colonies, OGS and YCH. Statistical significance of difference in the mean DV/D was tested by the Cochran-Cox method (S. Ishii 1983); and in the distribution patterns of the DA-, VA-, and DVP-types by the Chi-square test (Table 1).

The mean DV/D in the three colonies is similar to or larger than that of OGS, but far greater ($P < 0.01$) than that of YCH (Table 1). A difference in the mean DV/D among the three colonies is also recognized; the mean in TKU is significantly ($P < 0.01$) larger than that of TWMC, that of IMS being intermediate, and its variation range being widest in TKU and smallest in TWMC.

The distribution pattern of the DA-type is similar in TWMC and OGS, but different among others. TWMC and IMS share the same type of the VA- and DVP-type distribution and TKU is significantly different from TWMC and IMS in any genital arm types. Among the combinations of the DA-, VA-, and DVP-types, combinations (K, I)-(d, f) are prevalent in the three Horaana colonies, and (I, J)-g in OGS, while (D–F, H)-d in YCH (Table 2).

Table 2. Occurrence percentages >10% of the combinations of the DA-, VA- and DVP-types.

Sample	n	Combination	%
TKU (F_{167})	50	K-d-II	46.0
		I-d-II	24.0
		I-f-II[q]	12.0
		others[a]	18.0
TWMC (F_{173})	50	I-d-III	30.0
		I-f-II[q]	24.0
		I-d-II	18.0
		others[b]	28.0
IMS (F_{176})	46	I-f-II[q]	19.6
		I-e-III[q]	17.4
		I-d-III	10.9
		I-d-II	10.9
		others[c]	41.2
OGS	50	I-g-II[q]	68.0
		I-g-III[q]	14.0
		J-g-II[q]	10.0
		others[d]	8.0
YCH	27	F-d-III[p]	22.2
		E-d-II[p]	14.8
		H-d-II[p]	11.1
		D-d-II[p]	11.1
		others[e]	40.8

[a] Including 3; [b], 8; [c], 13; [d], 5; [e], 9 combinations other than the listed, [p], combinations common in *Cx. p. pallens*; and [q], those common in *Cx. p. quinquefasciatus*.

DISCUSSION

As discussed in previous papers (Ishii 1983a, 1983b) and others summarized in Table 3, the larval characters of the three Horaana colonies (formerly named as Kawasaki-1c (= F_{176}; IMS), -2c (= F_{174}; TWMC), and -3c (= F_{167}; TKU)], especially the siphon-head index, the shape of the siphon, the siphonal index, and the number of the branches of the siphonal setal tufts are beyond the range of *Cx. p. pallens* collected from central Japan but conform to that of *Cx. p. quinquefasciatus*.

The same conclusion is observed in the male genital characters studied above. The mean DV/D in TWMC and IMS is comparable with that of *Cx. p. quinquefasciatus* collected from the southern islands of Japan, but much different from *Cx. p. pallens* of central Japan (Ishii 1980). The extraordinarily large mean DV/D in TKU is puzzling. Since there are significant differences between TKU and the other two in the DA-, VA-, and DVP-type distribution (Table 1) and also in the combination of the three genital arm types (combination K-d prevails in TKU,

[2] Sohn, S. R. 1987. Morphological analysis of the *Culex pipiens* complex by typology of male genitalia. (in Korean, with English abstract) Dr.Sc. dissertation, Kyungpook National University, Korea.

Table 3. Comparisons of the larval characters among the Horaana and other strains (Ishii 1983a).

Sample		IMS (F_{176}) (Kawasaki-1c)	TWMC (F_{174}) (Kawasaki-2c)	TKU (F_{167}) (Kawasaki-3c)	OGS (Ogasawara-4)	YCH (Yachiyo-1)
	n	50	50	50	50	50
Siphon-Head index[a]	Mean ± SE	1.00 ± 0.01	1.02 ± 0.01	1.00 ± 0.01	1.00 ± 0.01	1.11 ± 0.01
	Range	0.92–1.09	0.88–1.12	0.94–1.09	0.92–1.08	0.90–1.24
Siphonal index[b]	Mean ± SE	3.76 ± 0.02	3.74 ± 0.03	3.61 ± 0.02	3.77 ± 0.03	4.15 ± 0.03
	Range	3.42–4.19	3.30–4.19	3.30–3.98	3.44–4.41	3.67–4.73
No. branches of 1st siphonal setal tufts[c]	Mean ± SE	8.72 ± 0.24	8.48 ± 0.20	12.36 ± 0.24	11.52 ± 0.28	6.20 ± 0.22
	Range	4–12	6–12	9–16	8–17	4–10

[a] = SL/HW, [b] = SL/SW, where SL is siphonal length, HW is head width, and SW is siphonal width; [c], a sum of both sides.

but I-d in TWMC and IMS), it is possible that TKU was reared under quite different conditions from the other two Horaana colonies.

Some similarities were observed in the mean DV/D and the DA-type distribution among the three Horaana colonies and OGS, but none at all in any character examined here between the former and YCH. Therefore, the Horaana males previously determined as *Cx. p. pallens* should be provisionally identified as *Cx. p. quinquefasciatus.*

The matter of the initial morphology of the Horaana strain (i.e., on discovery just over two decades ago) now must be considered. The only reported study of this reveals that its mean [100] D/V was 80.0 (n = 107) (= 0.125 in DV/D) with SD being 9.35 (no precise filial generation of the specimens and date of measurement given, but probably the F_{11} or earlier generation before or during March 1965) (Sasa et al. 1966). This certainly suggests that as originally encountered the subterranean Horaana strain fitted the criteria for *Cx. p. pallens*. It can only be concluded that as preserved in laboratory colonies since then, the strain has regressed to (or had become contaminated with the dominant) *Cx. p. quinquefasciatus.*

To explain this change, the following can be assumed:

1) Since laboratory-adapted mosquito stains are believed to maintain their characters with reasonable stability during a course of routine subculturing, provided that there was no intended selection pressure and that the strain has been maintained in impeccably supervised laboratories (such as the very well-equipped and staffed Japanese ones concerned in this instance), therefore, the original Horaana strain should be morphologically compatible with its descendants as examined by us. The first assumption is thus that the above mean DV/D (Sasa et al. 1966) is not reliable. 2) If the above value is reliable, then the second assumption is that the strain might have been erroneously handled (mislabelling, contaminated, etc.). 3) The third assumption is that the strain has evolved or regressed more rapidly than ever before reported. At the present stage, we cannot conclude which assumption is the most probable.

Some cases have been reported in which laboratory strains have changed their biological traits during culture. Wilton and Jakob (1985) reported temperature-induced morphological changes in hybrid strains of *Cx. pipiens*. An autogenous female spontaneously appeared from an unautogenous strain of *Cx. p. pallens* long maintained in the laboratory (Sasa et al. 1966). Stimulation with diet amino acids (Hosoi et al. 1975) has precipitated similar changes. Even though such cases were few and less drastic than the change in the Horaana strain, the third of the above alternatives seems most likely. This solution might also be supported by the fact that Horaana strain is inherently flexible in the characters discussed, because in 1981 TKU had already become quite different from IMS and TWMC even though they had been maintained under similar laboratory conditions without any intended selection pressure after their separation (at 25–26°C; Y. Kurihara and Y. Wada, personal communication).

In Japan, there has been a prevailing belief that *Cx. pipiens* group mosquitoes collected here (except in the southern islands) were automatically identifiable as *Cx. p. pallens* without detailed examination. This arose from the conviction that the Japanese *Cx. p. pallens* is "a homogenous group of organisms, morphologically intermediate between *pipiens* and *quinquefasciatus* forms" (Spielman 1967). The fact that the original females were collected from an overwintering site where *Cx. p. quinquefasciatus* had never been reported, might point to neglect of detailed morphological studies.

Should the characters of the Horaana strain prior to F_{11} prove to be the same as for those that we examined, earlier conclusions about this strain (Sasa et al. 1966, 1967) must be prudently reconsidered. To avoid difficulties such as discussed above, it should be pointed out the necessity of preserving voucher material when standard laboratory colonies are first established.

ACKNOWLEDGMENTS

We would like to express our sincere thanks to Dr. M. Laird, University of Aukland and Dr. A. R. Barr, School of Public Health, University of California, Los Angeles, for critical reading of the manuscript. Thanks are also extended to Drs. Y. Wada and A. Shirasaka, Department of Parasitology, Tokyo Women's Medical College, and Dr. T. Kurihara, Medical School of Teikyo University, for offering specimens and information, and to Dr. M. Ikeuchi, National Institute of Agricultural Science for making further specimens available. Also appreciation is expressed to the Ministry of Education, Science and Culture, Government of Japan for a research grant to the junior author while staying at the University of Tokushima.

REFERENCES CITED

Barr, A. R. 1986. Bases of mosquito systematics. J. Am. Mosq. Control Assoc. 2:261–266.

Hosoi, T., K. Utida, S. Sato and O. Matsumura. 1975. Initiation of egg development in the mosquito, *Culex pipiens pallens*, stimulated by diet amino acids. (in Japanese, with English summary). J. College Arts and Sciences, Chiba Univ. B-8:73–91.

Ishii, S. 1983. BASIC niyoru Tokeishori (PC-8801), Parametorikku oyobi Nonparametorikkuhou [Statistical Treatments in BASIC (PC-8801). Parametric and nonparametric methods] (in Japanese). Baifukan, Tokyo, vi+167 pp.

Ishii, T. 1980. On the *Culex pipiens* group in Japan Part III A historical review of its research 4. Review of the adult character (3). (in Japanese, with English abstract). J. Sci., Univ. Tokushima. 13:29–62.

Ishii, T. 1983a. On the *Culex pipiens* group in Japan Part III A historical review of its research 7. Review of the larval characters (3). (in Japanese, with English abstract). J. Sci., Univ. Tokushima 16:27–109.

Ishii, T. 1983b. Sketches of siphons of the 4th instar larvae of the *Culex pipiens* group. Akaieka Newslett. 7:21–23.

Sasa, M., A. Shirasaka and T. Kurihara. 1966. Crossing experiments between *fatigans*, *pallens* and *molestus* colonies of the mosquito *Culex pipiens* s.l. from Japan and southern Asia, with special reference to hatchability of hybrid eggs. Jpn. J. Exp. Med. 36:187–210.

Sasa, M., A. Shirasaka and T. Kurihara. 1967. Comparative studies on some morphological and physiological characters of the *Culex pipiens* complex of Japan and southern Asia. Jpn. J. Exp. Med. 37:475–504.

Spielman, A. 1967. Population structure in the *Culex pipiens* complex of mosquitos. Bull. W. H. O. 37:271–276.

Wilton, D. P. and W. L. Jakob. 1985. Temperature-induced morphological changes in *Culex pipiens*. J. Am. Mosq. Control Assoc. 1:174–177.