Kontyû, Tokyo, 49 (1): 37–44. March 25, 1981

The Sexcomb of *Drosophila simulans*: Geographical Variation and Genetic Analysis

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Synopsis The tooth number of sexcombs was examined, using many populations of *Drosophila simulans*. The tooth number of American populations was fewer than that of other populations. The crosses between high and low tooth number strains suggested that this morphological character was controlled by many genes (polygenic system) on autosomes without maternal effects or significant dominance. In addition, the tooth number of sexcombs was not significantly correlated with wing length or mating ability. The origin of Japanese *simulans* is discussed.

The sudden invasion of *Drosophila simulans* into Japan was reported by WATANABE and KAWANISHI (1976). This species had never been found in the mainland of Japan until a few reports (KIKKAWA & PENG, 1938; OKADA, 1956, 1971). One of our interests is to determine from which country this species has come to Japan. There are several ways to solve this problem. One is to compare some genetic characters of Japanese *simulans* with ones from other countries. We focused on the tooth number in sexcombs of males, since the variation of that character seemed to be genetically controlled. BOCK and WHEELER (1972) showed that the mean tooth number of sexcombs from an Australian population of *D. simulans* was 9.28 with the range of 7–11, whereas that of *D. melanogaster* from the same population was 9.68 with the range of 7–12. Recently, COOK (1977), using the technique of amputating the sexcombs from forelegs, suggested that the sexcombs of *Drosophila* were related to sexual behavior, particularly the grasping of females by males.

We report here geographical variation in the tooth number of sexcombs in populations of *D. simulans* and data suggesting that this morphological character is controlled by many genes without significant dominance. The relationships between this character and wing length or mating ability are also investigated from the viewpoint of adaptation. Finally, the origin of Japanese *simulans* is discussed.

Materials and Methods

Drosophila simulans: Many populations of *D. simulans* which have been sampled in different times from different countries of the world and from various localities in Japan were used in this study (see Table 2a and 2b). Samples from some populations have been maintained in the laboratory as isofemale lines, while samples from others were from wild flies which have been recently collected from natural populations.

Measurement of the tooth number of sexcombs: In order to estimate the mean tooth number of sexcombs for each population, 20 to 100 males were sampled from each population source. The tooth number of sexcombs was measured under a microscope (400X), using either one of the two forelegs. The genetic control of tooth number was analyzed by the following crosses: (1) Ten females sampled randomly from a high tooth number strain (Tananarive, mean tooth number ($\bar{x}$)=12.26) were crossed to ten males sampled randomly from a low tooth number strain (USA of unknown origin, $\bar{x}$=9.02). The reciprocal cross was also carried out. Ten pairs of the F_1 flies were crossed to each other to obtain F_2 offspring. (2) The USA and Tananarive strains mentioned above were crossed to two marker strains *bw* (2-not located); *st* (3–40), $\bar{x}$=11.23 and *pm* (2–103); *simA* (3-not located), $\bar{x}$=12.13. The marker strains with a recessive marker on each chromosome were also crossed to each other.

Wing length: Thirty two isofemale lines from each population of Mishima and Kokura were used for the measurement of wing length and tooth number. Five pairs of two days old females and males were kept in a vial. Vials having 50–100 F_1 pupae were chosen to standardize density effects. Five females and five males were sampled from each isofemale line. The right wing of females was removed and put on a slide glass with a drop of water and a cover slip. The length of the wing from the outer margin of the anterior crossvein to the tip of the wing along the third longitudinal vein was measured. The right legs of males (brothers) were taken to measure the tooth number of sexcombs.

Mating ability: The mating abilities of a high tooth number strain (Naze, $\bar{x}$=11.70) and a low tooth number strain (Chapel Hill, $\bar{x}$=9.92) were tested as follows: Ten pairs (two days old) of flies were crossed within each strain. The number of pairs which were mating in the vial was directly observed every five minutes. The examination was replicated ten times for each strain.

Results

(1) *Comparison in the tooth number of sexcombs between wild males and laboratory males*: As shown in Table 1, the tooth number of USA *simulans* (9.80 and 9.92) was significantly fewer than that of Japanese one (10.79 and 10.80). However, no significant differences in the tooth number of sexcombs between wild caught males and laboratory males which had been kept as isofemale lines were detected for both populations. This suggests that the character is stable to environmental variables.

(2) *Geographical variation of the tooth number of sexcombs*: The tooth number of sexcombs was surveyed for various populations in the world. The data are listed in Table 2a. Although the tooth number of sexcombs of individual values extends from 7 to 15, the mean tooth number of each population ranged from 9.02 to 12.26,

Table 1. Comparison in the tooth numbers of sexcombs between wild caught flies and laboratory flies maintained as isofemale lines sampled in the same population of *D. simulans*

Population	Wild		Isofemale	
	N*	Mean SE**	N*	Mean SE**
Chapel Hill (USA), 1977	50	9.80±0.15	50	9.92±0.16
Kokura (Japan), 1976	50	10.80±0.17	32×5	10.79±0.12

* Number of males tested; ** standard error.

with an average 10.48. The USA populations carried generally fewer teeth (9.70 with the range of 9.02–10.00) than the other populations. The tooth number of the geographical strains did not correlated with the latitude of strain origin, suggesting that this morphological character is not affected by temperature. The average tooth

Table 2a. The tooth number of sexcombs in geographical populations of *D. simulans*

Population	Latitude	N*	Mean	SE**	Range
I. Africa					
Tananarive (Madagascar)[a]	19.5S	50	12.26	0.15	10–15
Tenerife (Canary Is.)[a]	28.2N	50	11.50	0.13	10–13
Congo (unknown origin)[a]	—	50	10.96	0.15	9.13
St. Denis (Reunion I.)[a]	20.5S	50	10.94	0.14	8–13
Alexandria (Arab)[a]	31.1N	50	10.48	0.13	9–12
Brazzaville (Congo)[a]	4.2S	50	10.32	0.15	8–13
Las Palmas (Canary Is.)[a]	28.0N	50	10.16	0.15	8–13
(Mean)			(10.95)		
II. Australia					
Sydney[b] (50)	33.5S	50	10.80	0.24	9–13
Melbourne[b] (40)	37.5S	40	10.65	0.16	9–13
(Mean)			(10.73)		
III. Europe					
Villeurbanne (France)[a]	45.5N	50	10.78	0.15	9–13
Almeria (Spain)[a]	36.8N	50	10.76	0.22	9–13
Toulon (France)[a]	43.1N	50	10.64	0.14	9–13
London (England)[c]	51.5N	50	10.62	0.13	7–12
(Mean)			(10.70)		
IV. America					
Brownsville (TX), 1978 (50)[b]	25.5N	50	10.00	0.15	7–13
Chapel Hill (NC), 1977 (50)[b]	35.8N	50	9.92	0.16	8–13
Hawaii[a]	25.1N	50	9.86	0.11	8–13
Chapel Hill (NC), 1977[c]	35.8N	50	9.80	0.15	8–13
Austin (TX), 1977[b] (10)	30.5N	50	9.58	0.14	7–12
USA (unknown origin)[a]	—	50	9.02	0.12	8–11
(Mean)			(9.70)		

* Number of males tested; ** standard error; [a] one laboratory stock kept in mass culture; [b] isofemale lines (The number in parenthesis represents the number of isofemale); [c] wild caught males.

Table 2b. The tooth number of sexcombs in Japanese populations of *D. simulans*

Population		N*	Mean	SE**	Range
I.	Eastern:				
	Mishima, 1976[b] (32)	32×5	10.65	0.11	8–13
	Mishima, 1975[b] (20)	20	10.55	0.20	9–12
	Mishima, 1977[b] (50)	50	10.45	0.25	9–14
	Fujinomia[d]	20	10.35	0.27	8–12
	Maebashi[c]	20	10.30	0.26	9–12
	Omiya[c]	20	10.30	0.24	8–12
	Tokyo[c]	20	10.20	0.27	8–13
	Omiya[d]	20	10.15	0.27	8–12
	Utsunomiya[d]	20	10.10	0.21	8–11
	Sagamihara[c]	20	10.05	0.26	8–12
	Naoetsu[d]	20	9.80	0.21	8–11
	Shimoda[d]	20	9.80	0.17	9–11
	Tomizawa[d]	20	9.10	0.19	8–11
	(Mean)		(10.14)		
II.	Ogasawara:				
	Ogasawara[a]	50	10.46	0.15	9–13
	Ogasawara, 1973[c]	100	10.52	0.10	8–13
	(Mean)		(10.49)		
III.	Western:				
	Saiki[d]	20	11.60	0.25	10–14
	Masaki[c]	20	11.10	0.32	9–15
	Tosu[c]	20	11.00	0.25	9–14
	Saiki[c]	20	10.90	0.23	9–13
	Kumamoto[d]	20	10.80	0.29	9–13
	Izumi[d]	20	10.80	0.19	9–12
	Kokura[c]	50	10.80	0.17	8–13
	Kokura, 1976[b] (32)	32×5	10.79	0.12	8–14
	Kagoshima[d]	20	10.75	0.22	9–12
	Kochi[c]	20	10.70	0.26	9–13
	Miyazaki[c]	20	10.55	0.17	9–12
	Masaki[d]	20	10.55	0.18	9–12
	Shimonoseki[c]	20	10.50	0.26	9–12
	Kagoshima[c]	20	10.45	0.20	9–12
	Hiroshima[d]	20	10.40	0.26	9–14
	Sukumo[d]	20	10.35	0.17	9–11
	Miyazaki[d]	20	10.30	0.18	9–12
	(Mean)		(10.73)		

*, **, [a], [b] and [c]: see the footnotes of Table 2a; [d] several females origin.

number of Japanese *simulans* was 10.47, including 10.49 for an Ogasawara population (see Table 2b). The tooth numbers were, on average, fewer in eastern populations of Japan (10.14) than in western ones (10.73), which were more similar to those of European (10.70), Australian (10.73) and African (10.95) populations than to those of American ones (9.70).

(3) *Genetic analysis of the tooth number of sexcombs*: The results of genetic analysis of the tooth number of sexcombs are shown in Table 3. Reciprocal crosses did not show any differences in the tooth number (see Crosses I and II in Table 3). The mean scores of F_1 (11.04 and 11.12) and F_2 (10.76 and 10.86) were close to the midparent value (10.64) calculated from each parental score. The similar situation was observed in the other crosses. The variances of F_1 in Crosses I and II were smaller than those of F_2 in these crosses, respectively. This suggests that the tooth number is controlled by many genes (polygenic system) on autosomes without maternal effect or significant dominance.

Table 3. Means ($\bar{x}$) and their standard errors (SE) of the tooth number of sexcombs in F_1 and F_2 obtained from the crosses of four strains

	N*	$\bar{x}$	SE
Parental strains			
USA strain (Low)	50	9.02	0.12
Tananarive strain (High)	50	12.26	0.15
bw; *st* strain	30	11.23	0.20
pm; *simA* strain	30	12.13	0.14
F_1			
Low ♀♀ × High ♂♂ (Cross I)	50	11.04	0.14
High ♀♀ × Low ♂♂ (Cross II)	50	11.12	0.12
bw; *st* ♀♀ × Low ♂♂ (Cross III)	30	10.50	0.14
bw; *st* ♀♀ × High ♂♂ (Cross IV)	30	11.77	0.17
pm; *simA* ♀♀ × Low ♂♂ (Cross V)	30	10.93	0.14
pm; *simA* ♀♀ × High ♂♂ (Cross VI)	30	12.53	0.14
pm; *simA* ♀♀ × *bw*; *st* ♂♂ (Cross VII)	30	11.70	0.16
bw; *st* ♀♀ × *pm*; *simA* ♂♂ (Cross VIII)	30	11.73	0.16
F_2			
Cross I	50	10.76	0.16
Cross II	50	10.86	0.17

* Number of males tested.

(4) *Correlation between the tooth number of sexcomb and wing length*: Using 32 isofemale lines from each of two Japanese populations, the tooth number of sexcombs was compared with the wing length in each population. As shown in Table 4, the correlations between these two characters were not significant (r=0.027, d.f.=

Table 4. Correlation between tooth number of sexcomb and wing length in 32 isofemale lines of two Japanese populations in *D. simulans*

Population	Wing length[a]	Tooth number	r^b (d.f.=30)
	Mean SE[c]	Mean SE[c]	
Mishima, 1976	1220.22±5.11	10.65±0.11	0.027[ns]
Kokura, 1976	1220.28±4.29	10.79±0.12	0.077[ns]

[a] Arbitrary unit; [b] coefficient of correlation between two characters; [c] standard error; [ns] not significant.

30 for Mishima; r=0.077, d.f.=30 for Kokura). Analysis of variance was carried out for each character. The analysis showed that there were no significant differences in both characters between the two populations, although there were significant differences between isofemale lines of each population.

(5) *Mating ability of high and low tooth number strains*: Mating ability of high (Naze) and low (Chapel Hill) tooth number strains was examined. The results are illustrated in Fig. 1. The curve of mating ability on time (every five minutes) for a high tooth number strain was similar to that for a low tooth number strain. It means that the variation of the tooth number of sexcomb did not affect the copualtion of flies.

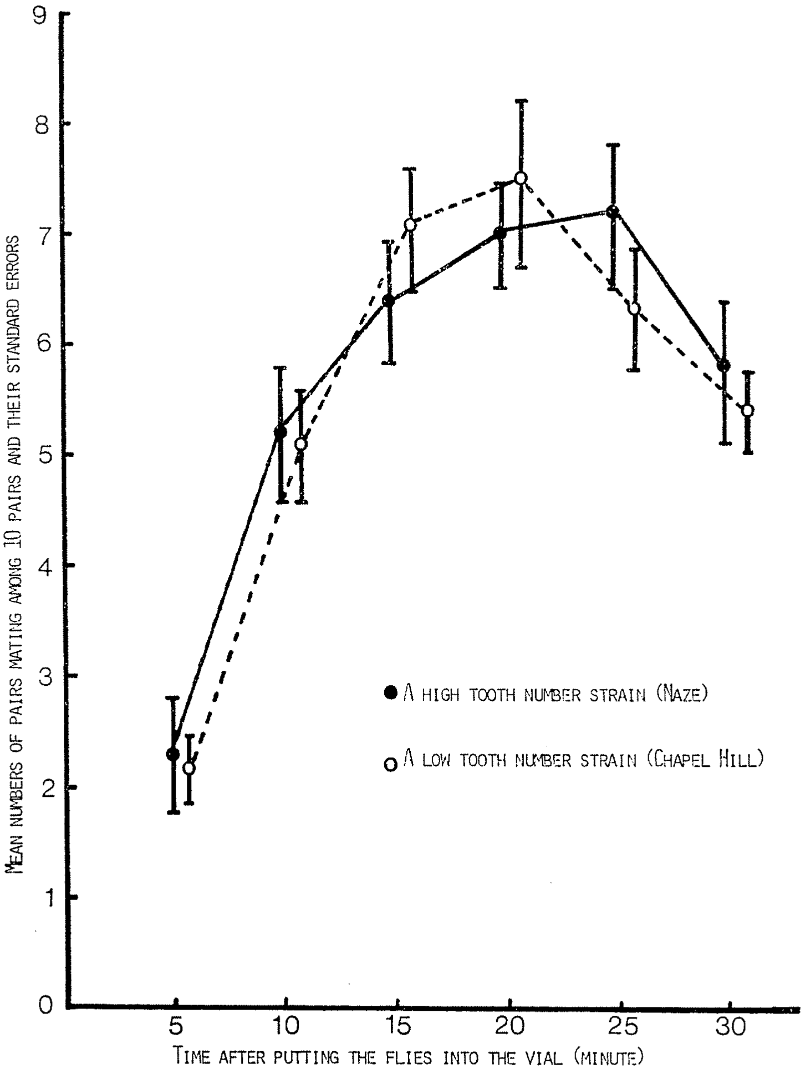

Fig. 1. Mating ability of high (Naze) and low (Chapel Hill) tooth number strains in *D. simulans*.

Discussion

The tooth number and the arrangement in *Drosophila* sexcomb have been used as a key character for the classification of the species. The present results clearly showed that the variation of the tooth number among geographical populations of *D. simulans* is genetically controlled. The character seems to be controlled by many genes (polygenic system) on autosomes without maternal effects and significant dominance. The geographical variation of this morphological character was not associated with latitude where the flies were collected. This suggests that tooth number is not easily modified by temperature conditions. The stable penetrance of this character was confirmed, using wild caught flies and laboratory cultures from the same natural populations. In addition, we could not detect any correlations between tooth number and other traits (wing length and mating ability) in this study. This seems to be no pleiotropic effects of genes controlling the tooth number on such traits. Though COOK (1977) reported that mating behavior was affected by no teeth in sexcombs, the tooth number variation for individuals (for instance 7 to 15) seems to have no associations with mating ability estimated by our method. In other words, though mating of males having the teeth in sexcombs would be normal, males without teeth falls the grasping in mating.

From the studies on the tooth number of sexcombs, we showed that a western population in Japan might be different from an eastern one in Japan, suggesting that the origin of the two populations of Japan may be different. The two populations of Japan were not similar to American ones, but a western population was similar to European, African and Australian ones. Although there is a possibility that Japanese *simulans* came from Europe, Africa or Australia but not from America, we do not have any evidence, supposing no founder effects at invading of flies, that Japanese *simulans* came from any of the former three regions. In addition, the data of the sexcombs demonstrated that an island population of Ogasawara, in which *D. simulans* has been found since 1936 (KOMAI, 1937), seemed to be slightly different from mainland (eastern and western) populations of Japan. WATADA *et al.* (1978) reported that the Ogasawara population was different from mainland population of Japan based on a comparison of gene frequencies at several allozyme loci (*Est-6*, *Est-C* and *Acph-1*) of this species. Thus, the origin of *D. simulans* in the mainland of Japan seems to be neither America nor Ogasawara Island, but we do not know its origin in more detail at present.

Acknowledgements We would like to thank Drs. T. K. WATANABE, National Institute of Genetics, Japan, R. A. VOELKER and W. SHERIDAN, NIEHS, USA, for their advice and critical reading of the manuscript. We also thank Drs. O. KITAGAWA, Tokyo Metropolitan University, Japan, J. DAVID, CNRS Gif-sur-Yvette, France, P. A. PARSONS, La Trobe University, Australia and M. J. TODA, Hokkaido University, Japan for providing the stocks of *D. simulans*.

References

BOCK, I. R., & M. R. WHEELER, 1972. The *Drosophila melanogaster* species group. *Studies in Genetics VII. Univ. of Texas Publ.* (7213): 1–102.

COOK, R. M., 1977. Behavioral role of the sexcombs in *Drosophila melanogaster* and *D. simulans*. *Behav. Genet.*, **7**: 349–357.

KIKKAWA, H., & R. T. PENG, 1938. Drosophila species of Japan and adjacent localities. *Jap. J. Zool.*, **7**: 507–552.

KOMAI, T., 1937. Collection of *D. simulans* from Japan. *Drosophila Inf. Serv.*, **8**: 78.

OKADA, T., 1956. Systematic Study of Drosophilidae and Allied Families of Japan. 184 pp. Gihôdô, Tokyo.

OKADA, T., 1971. Taxonomic and ecological notes on the Drosophilidae of the Bonin Islands (Diptera). *Proc. Jap. Soc. Syst. Zool.*, **7**: 67–73.

WATADA, M., Y. N. TOBARI, & S. OHBA, 1978. Isozyme polymorphisms in natural populations of *D. simulans* and *D. melanogaster*. *Jap. J. Genet.*, **53**: 458.

WATANABE, T. K., & M. KAWANISHI, 1976. Colonization of *Drosophila simulans* in Japan. *Proc. J. Acad.*, **52**: 191–194.