

GEOGRAPHIC VARIATION IN THE INTRINSIC INCUBATION
PERIOD : A PHYSIOLOGICAL CLINE IN THE EMMA
FIELD CRICKET
(ORTHOPTERA : GRYLLIDAE : *TELEOGRYLLUS*)*

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INTRODUCTION

The seasonal regulation of development is an essential feature of adaptation in most species of insects inhabiting the temperate or cold regions. The chief physiological mechanism of this adaptation is diapause (ANDREWARTHA, 1952 ; DANILEVSKY, 1961 ; LEES, 1955, 1962 ; DE WILDE, 1962). The ability of more or less precise timing of diapause in diversified climates is presumably one of the most important factors determining the geographic range of a species. A species which thrives in extensive areas must be equipped with a timing device which works efficiently in various conditions in different parts of its range. Such a geographic adjustment would be achieved to some extent by a phenotypic modification of the response controlling the evocation of diapause (GORYSHIN, 1955 ; GEYSPITS, 1960 ; cited in DANILEVSKY). For further extension of the range beyond the limits of this phenotypic plasticity, genetic differentiation of local populations should occur. Evidence suggests that such a variation is in fact of common occurrence in insects. Most outstanding examples are the regular increment in the critical length of the diapause-inducing photoperiod with increasing latitude of the habitat, as found in several species of the Lepidoptera (DANILEVSKY, 1957a, b, 1961). Other instances are also known in which the incidence of diapause shifts from a facultative to an obligatory nature (PREBBLE, 1941a ; LE BERRE, 1951, 1953, 1959 ; DANILEVSKY, 1961). These two facets of the variability in photoperiodic response are often interrelated. Thus in *Ostrinia nubilalis* the incidence of diapause, as measured in several constant conditions of light and temperature, varies among local populations and is correlated with the latitude of the population source (BECK and APPLE, 1961).

This way of geographical adaptation is neither available nor required for those species which are strictly univoltine, entering diapause in each generation irrespective of the day-length, temperature, food or other external stimuli. Yet their rates of development and diapause completion are in most cases influenced by temperature, so that a certain kind of geographic adaptation is still required by them in order to harmonize the life cycle with the seasonal changes of different climates. The intrinsic variability in the rates of development and diapause completion might conceivably be one of the possible ways of such adaptation. GOLDSCHMIDT (1932, 1938) showed that there were genetic variations among local populations of the univoltine moth, *Lymantria dispar* :

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the hatching time of its egg after hibernation varies in such a way that the larva appears at the local season when adequate food is available. This adjustment of hatching time is doubtless due to the variable intensity of the egg diapause. Similar adaptive variations seem to occur in the peculiar annual cycle of the winter moth, *Operophtera brumata*; WYLIE (1961) elucidated that there were intrinsic local differences in the incubation period as well as in the developmental period of the pupa. Again, ANDREW-ARTHA (1952) found that the western and southern races of the univoltine grasshopper, *Austroicetes cruciata*, differed in their requirements for temperature during diapause development of the eggs.

The Emma field cricket is also a typical univoltine insect, showing local differentiation in its physiological traits. It has but one generation a year due to an obligatory diapause in the egg stage throughout the Japanese archipelago. In these parts of its range, there is a steep geographic gradient of climatic conditions. The annual mean temperature ranges from 7°C in the north (Sapporo) to 19°C in the south (Tanegasima). Both the duration of the season and the amount of temperature accumulation available for growth rapidly decrease northward. Therefore, this species tolerates a considerably wide range of climatic conditions even in the narrow extent of this country.

The results of previous observations suggest that despite the invariable voltinism this field cricket shows a conspicuous adaptive variation (MASAKI, 1963). A physiological difference has thus been found between two local samples which had been derived, respectively, from the northern and southern parts of this country. When compared under the same conditions in the laboratory, the northern population completed its life cycle in a much less time than did the southern one, owing to the briefer diapause and the more rapid rate of development. BIGELOW (1960b) found a similar difference between the southern and northern populations of *Gryllus veletis* and *Gryllus pennsylvanicus*, respectively. These local characteristics are presumably controlled by genetic factors.

If the Emma field cricket exclusively feeds upon a particular kind of plant which is available only at a limited period of the year, the food supply must be considered first of all as an important factor involved in the natural selection of the local characteristics. The species, however, is omnivorous. The details of its food habit in the field are unknown, but this cricket is found in various habitats such as crop fields, riversides, grassland, gardens and so on. Occasionally it causes serious damage to vegetable crops, especially seedlings of cruciferous and leguminous plants and also devours the fruits of tomatoes and cucurbits. When reared in the laboratory, the species feeds on remarkably variable kinds of food, cucumber, pumpkin, apple, several kinds of grass and weeds, wheat flour, rice bran, dry fish, skim milk and many others. It is unlikely that the food supply is a decisive factor limiting the seasonal activity of this cricket.

It seems then probable that the physiological traits of the local populations are directly subject to climatic selection. If so, the variation would show a consistent geographic tendency and would be correlated closely with some climatic factor or factors. Based on this assumption, the present work has been carried out in order to elucidate the geographic tendency of this adaptive variation along a stretch of about 2,000 km in the Japanese archipelago. The physiological trait to be dealt with here concerns with the incubation period. It is hoped that the results would provide some information pertaining to the geographic adaptation, distribution or divergence of this species.

MATERIAL AND METHOD

Collecting sites. Several adults or late instar nymphs of the Emma field cricket were collected from different areas covering almost the whole range of the country. The collecting sites are shown on the map in figure 1 and listed in table 1. The latitude, longitude and altitude of each collecting site were read by interpolation on the 1 : 50,000 topographical map published by the Kokudotiriin (the Geographical Survey Institute of Japan). Since the map is marked with contour lines at 20-meter height intervals, the altitude of the place could not be determined accurately by this method ; approximations with errors less than ± 10 m would, however, suffice for the present purpose. The climatic conditions were cited from the Climate of Japan (edited by K. WADATI, 1958), the volumes 4 and 5 of the Temperature of Japan, or the 1958 edition of the Rikanenpyo, all compiled and published by the Tokyo Meteorological Institute.

General procedure. The eggs laid by the field-collected insects were designated

Table 1. *Geographic location and annual mean temperature of the places from which strains of the Emma field cricket were collected. See also the map in figure 1.*

Locality	Latitude (°N)	Longitude (°E)	Altitude (m)	Temp. (°C)
1. Sapporo	43.07	141.32	10	7.4
2. Hakodate	41.73	140.78	10	8.5
3. Aomori	40.78	140.78	10	9.2
4. Iwaki	40.62	140.32	290	*
5. Hirosaki	40.58	140.48	50	10.8
6. Owani	40.50	140.57	230	*
7. Akita	39.73	140.10	10	10.5
8. Morioka	39.72	141.10	130	9.3
9. Omagari	39.47	140.48	30	10.3
10. Tono	39.33	141.50	290	9.8
11. Mizusawa	39.03	141.07	150	10.3
12. Sakata	38.92	139.83	10	11.5
13. Sinzyo	38.77	140.28	90	11.4
14. Atsumi	38.62	139.58	10	12.7 a)
15. Siogama	38.32	141.01	30	11.1 b)
16. Sendai	38.25	140.85	50	11.1
17. Yamagata	38.25	140.32	130	10.7
18. Arakawa	38.13	139.47	10	12.4 c)
19. Yonezawa	37.92	140.10	250	11.3
20. Niitsu	37.80	139.10	10	13.1
21. Hukusima	37.75	140.47	70	12.0
22. Haranomati	37.63	140.97	30	12.3
23. Taira	37.05	140.87	30	13.5
24. Nakano	36.77	138.37	350	11.7
25. Toyama	36.68	137.20	10	13.0
26. Sinonoi	36.57	138.17	390	12.0
27. Takayama	36.13	137.27	590	9.9
28. Tokyo	35.68	139.67	30	14.3
29. Yonago	35.43	133.33	10	13.8
30. Inatake	35.25	137.50	530	11.9
31. Tsu	34.75	136.50	10	14.6
32. Nisinomiya	34.75	135.37	10	15.3 d)
33. Okayama	34.68	133.91	10	14.6
34. Taizi	33.58	135.95	50	16.5 e)
35. Hukuoka	33.58	130.37	10	15.1
36. Koti	33.55	133.68	10	15.6
37. Miyazaki	31.90	131.41	10	16.6

a) - annual mean temperature at Nezugaseki, b) at Sendai, c) at Murakami, d) at Osaka, e) at Sionomisaki.

* no data.

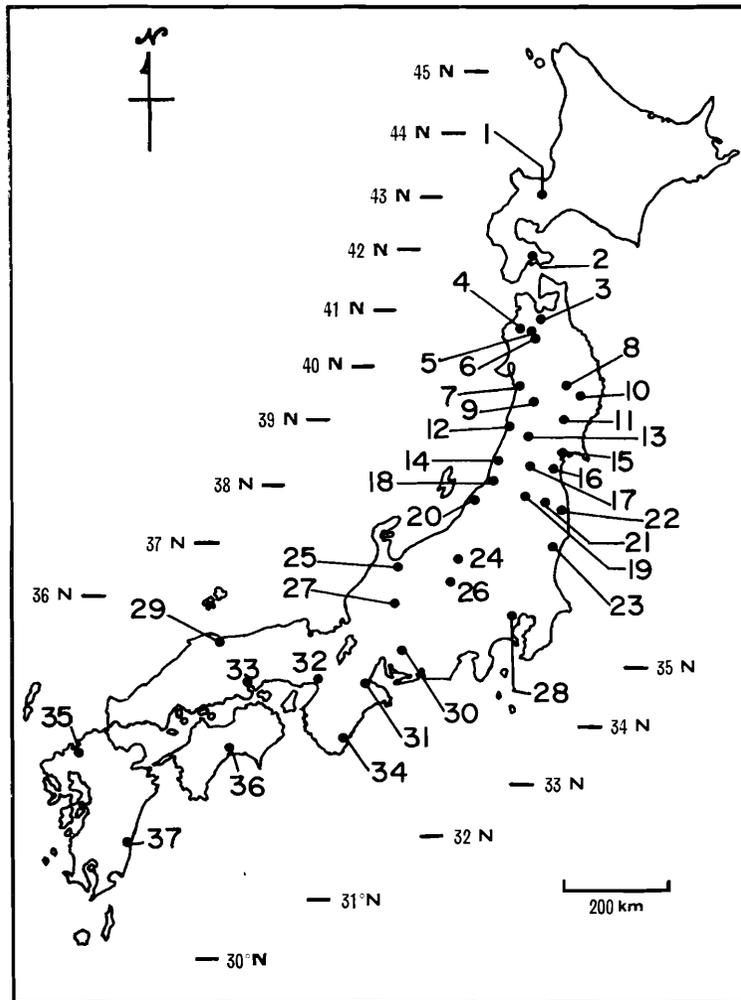


Fig. 1. Map showing the localities from which the stocks of the Emma field cricket were collected for this study. The locality names are given in table 1.

as the first generation. In order to secure successive generations, they were held at a high temperature during the pre-diapause incubation, and then kept in a cold storage for more than four months. This period of chilling was sufficient to break diapause in all local strains examined. After chilling, they were returned to 25°C for hatching. Nymphs were reared at 25°-30°C or at the room temperature in summer. Adults were kept at the same temperatures for about one to three weeks before they were allowed to oviposit. Dishes of moist sand were then introduced into the rearing jars and a large number of eggs were laid in the sand within a few days. Eggs of uniform age were thus obtained. RAKSHPAL (1962) recently found that in *Gryllus pennsylvanicus* the age of parents influenced the intensity of diapause of their progeny. About this matter nothing was known when the work was undertaken and no special care was taken to obtain eggs from adults of a uniform age. The first generation eggs were deposited by

wild females of unknown age, and also the age of the parents was variable in the subsequent generations. This might account for some fraction of the experimental errors, though the local strains were treated in similar manners.

The eggs under observation were kept either on moist filter paper and cotton wool or in moist sand in petri-dishes. In the former case, the eggs were counted and treated as lots of 100 and each treatment was usually comprised of two or more such lots. In view of the great labour of handling many local strains, this procedure was abandoned in later experiments, in which the eggs were left undisturbed in the moist sand in which they were laid. The dishes of sand were removed from the rearing jars and placed at desired temperatures for observation. This made the number of eggs per treatment very variable.

Every local sample was not available for every series of observations, because of the limited space for breeding the crickets. Several local stocks were maintained for three generations and subjected to experiments in each generation, others were discarded after two generations and still others were examined only in either the first or the second generation. In certain stocks, there was a tendency of decreasing viability during the successive laboratory breeding. It was also impossible to obtain many local specimens in the same season, and collecting trips were made several times in late summer or early autumn from 1958 to 1963 inclusive. Observations on the available strains were therefore not undertaken simultaneously, but several groups of local strains were examined separately. Each series of test was, however, consisted of samples collected at places widely apart from each other in order to discern the definite local differences among them. A similar geographic trend was repeatedly found in different series of observations so that the data were compiled on the basis of generation. The general tendency was more easily seen by this arrangement than by presentation of the data as separate series. It was confirmed that this compilation did not cause any substantial change of the conclusion.

Temperature treatments. In order to determine the local characteristics, the eggs of different local strains were subjected to various conditions of temperature. They were constantly kept at 20°, 25° or 30°C and the number of hatching nymphs recorded daily. At a temperature of 15°C most eggs of every local strain failed to hatch normally so that the duration of the incubation period could not be determined. The eggs were, however, examined for development at four-week intervals during a period of nine months at this temperature. Embryos that had completed revolution were easily identified when the eggs were covered with water in a glass dish and observed under a dissecting microscope (BROOKES, 1952). Their pigmented eyes were also recognizable by the naked eye. The number of such eggs were counted and recorded to compare the competence of different local strains to develop at this critical temperature.

The eggs of the Emma field cricket terminate diapause at a cold temperature as well, as do those of some other species of field crickets (BROWNING, 1962a, b ; HOGAN, 1960a ; RAKSHPAL, 1962 ; MASAKI, 1960). In order to evaluate the time required for breaking diapause under such conditions, several batches of 100 or 150 eggs of each local stock were exposed to 5-10°C for various periods. They were then incubated at 25°C and the percentages of eggs that hatched without delay were recorded. From these data the median effective durations of cold exposure for breaking diapause were obtained. The response of eggs to a still lower temperature of $0 \pm 1^\circ\text{C}$ was also tested by a similar method, though the median effective duration of exposure could not be estimated as referred to later on.

RESULTS

At 20°C. When the eggs of the Emma field cricket were constantly kept at temperatures above the developmental threshold, they developed for a few days and reached the so-called dumb-bell shaped stage (UMEYA, 1946), at which diapause obligatorily supervenes in all the local stocks examined. The diapause came to its end after a longer or shorter period of time without any exposure to a cold temperature except in certain small proportions of the viable eggs. Therefore, the duration of the egg stage could be measured at a constant warm temperature.

The results thus obtained at 20°C for the three laboratory generations are set out in table 2. As is usual with the diapause eggs kept under these conditions, nymphs hatched from them for a long period of time in each local strain. The standard deviation of the mean hatching time was considerably great. The hatching, however, was not necessarily sporadic and the mode of hatching time could be recognized in most cases as illustrated by examples of hatching curves in figure 2. Therefore, the durations of the incubation period of the local crickets might be compared in terms of the mean. Such comparisons reveal that there are considerable local differences.

In the first generation, the eggs from Sapporo, Hokkaido, hatched in the shortest period of 105 days while those from Koti, the southern coast of Sikoku, in the longest period of 193 days. These extremes were connected by graded series of other local strains. The mean duration of the egg stage at 20°C thus tended to be longer from

Table 2. *The durations of the incubation period at 20°C in three generations of different local strains of the Emma field cricket. The strains are arranged in the order of their original latitude from north to south.*

Strain	\bar{x}	<i>s</i>	<i>n</i>	Strain	\bar{x}	<i>s</i>	<i>n</i>
(First generation)							
Sapporo	105	11.4	291	Niitsu	117	23.0	158
Hakodate	119	26.5	224	Taira	173	31.0	78
Hirosaki	114	17.8	322	Nakano	120	22.0	191
Akita	107	10.6	173	Toyama	121	16.8	228
Omagari	113	7.9	74	Sinonoi	119	15.0	273
Tono	112	18.0	195	Tokyo	161	22.4	147
Mizusawa	127	25.0	199	Tsu	162	33.2	88
Sendai	185	34.6	382	Nisinomiya	149	21.9	222
Yamagata	163	35.8	1722	Okayama	168	24.0	79
Arakawa	151	33.2	1147	Koti	193	22.2	134
Yonezawa	147	33.5	1940				
(Second generation)							
Sapporo	101	11.0	192	Taira	201	54.6	644
Hirosaki	117	15.0	202	Nakano	115	15.8	409
Akita	119	15.0	194	Toyama	127	24.0	188
Tono	111	15.4	1370	Tokyo	151	32.0	178
Mizusawa	114	13.1	259	Tsu	167	34.0	189
Siogama	142	31.9	886	Okayama	171	29.0	186
Niitsu	138	32.0	444	Koti	219	36.0	157
(Third generation)							
Sapporo	101	12.4	900	Inatake	145	31.8	812
Hirosaki	107	17.5	1270	Tsu	185	56.4	740
Akita	130	28.7	580	Okayama	208	56.6	769
Toyama	114	20.9	226	Taizi	187	61.5	108
Sinonoi	145	32.7	680	Koti	206	58.4	475
Tokyo	183	48.8	485				

\bar{x} = mean duration of the egg stage in days ; *s* = standard deviation ; *n* = number of nymphs that hatched.

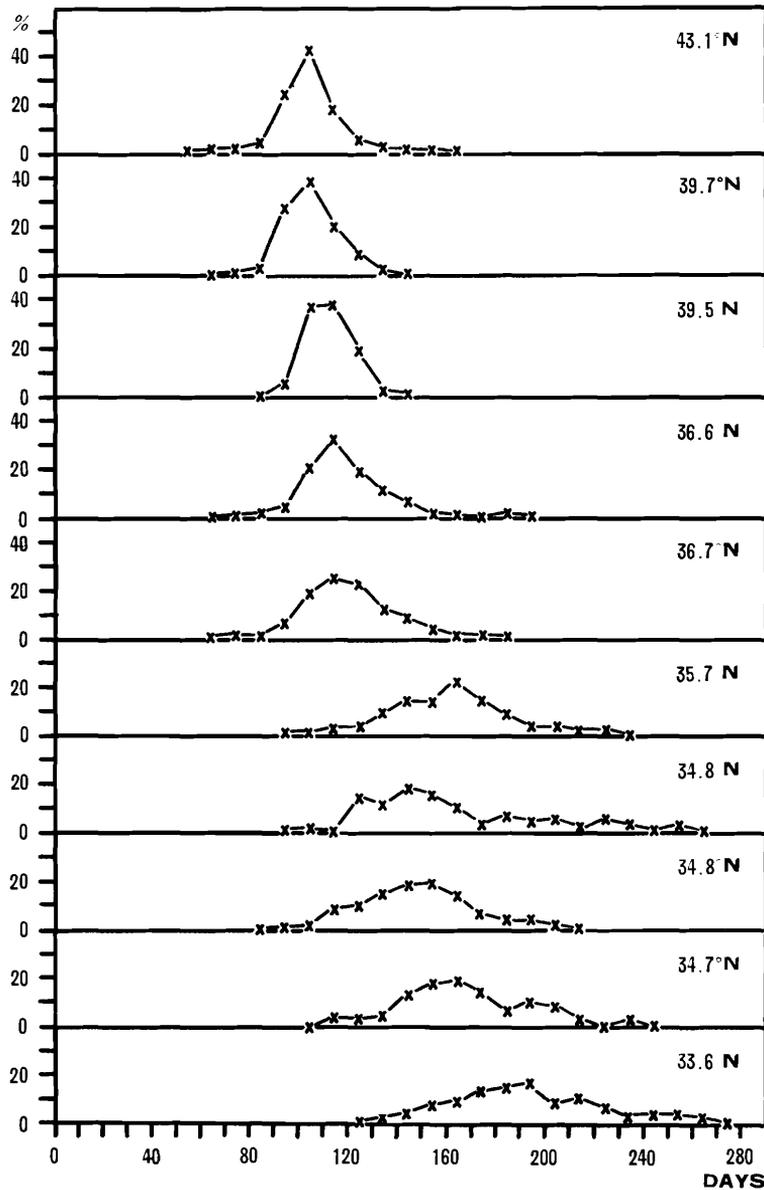


Fig. 2. Hatching curves of different local strains of the Emma field cricket at 20°C. From top to bottom, Sapporo, Akita, Omagari, Sinonoi, Toyama, Tokyo, Tsu, Nisinomiya, Okayama and Koti.

north to south, though there were considerable deviations from this general tendency. In the second generation, the northernmost eggs again took the shortest time to hatch while the southernmost ones the longest time. The general geographic trend was essentially the same as in the parents. The same was also true in the third generation. The eggs derived from Hokkaido or northern Honsyu hatched on the average in less than

four months, those from the central parts in four to five months and those from the southern parts took six to seven months to hatch.

Comparison between the results obtained with different generations may provide information about the stability of the incubation period of each local strain in these experiments. In some strains the incubation period was fairly constant throughout the experiments ; for example, the mean fluctuated only between 101 and 105 days in the Sapporo strain. In others the fluctuation was remarkable ; thus in the Okayama strain the mean incubation period ranged from 168 to 208 days. Several reasons might be considered for such fluctuations : variations in age or physiological state of the parents, unconscious failure in controlling the environmental conditions, genetic drift caused by the small number of specimens in the laboratory breeding, or selection operated under the artificial conditions of cultures. The primary purpose of the present work is, however, to elucidate the general geographic pattern of the variation but not to scrutinize minor differences between strains. The data described above seem to offer the required information.

At 25°C. A larger number of local strains were available for the experiments at 25°C, the results of which are compiled in table 3. From this table it is clear that every strain responds to the increase in temperature in a similar manner. The incubation period was on the average shortened by about one to three months at 25°C among different strains as compared with that at 20°C. The standard deviation for the mean was correspondingly decreased.

In the first generation, the mean incubation period varied from 80 to 125 days among different local stocks, and the northern ones tended to hatch earlier than the southern ones. The general geographic tendency was very similar to that observed previously, though the sequence of hatching of the local stocks was not precisely the same as at 20°C. In the second generation, the extremes of the local means were 74 and 134 days, respectively, and the southern strains were again preceded by the northern ones in hatching. In the last generation, the eggs of Sapporo and other northern areas hatched in the shortest period of 75 days while those from the Inland Sea coast (Okayama) and from the southern tip of the Kii peninsula (Taizi) did so in the longest period of 113 days.

The mean incubation period for each local stock fluctuated from generation to generation, as encountered in the experiments at 20°C. In particular, about half of the local strains examined in the last generation took somewhat shorter periods of time for hatching than in the preceding generations. Despite such fluctuations of ambiguous nature, the regularity in the geographic variation was still obvious through the successive generations at this temperature.

At 30°C. The response of local eggs to a still higher temperature was also tested on a small scale. Several local samples of the first generation were incubated at 30°C and the results obtained were given in table 4. These results should be compared with those obtained with the same generation eggs at 20° or 25°C. Such comparisons indicate that in most strains the mean length of incubation was shorter at 30°C than at 25° or 20°C. The increase in temperature from 25° to 30°C was, however, less effective than the increase from 20° to 25°C for shortening the incubation period. Furthermore, the eggs suffered higher mortality at 30°C than at the lower temperatures (MASAKI, 1960). It seems that this temperature is near the upper threshold for normal development of the diapause eggs.

Even at this high temperature persisted the same geographic tendency as repeatedly

Table 3. *The durations of the incubation period at 25°C in three generations of different local strains of the Emma field cricket.*

Strain	\bar{x}	<i>s</i>	<i>n</i>	Strain	\bar{x}	<i>s</i>	<i>n</i>
(First generation)							
Sapporo	83.6	10.4	2094	Yamagata	92.3	14.9	1040
Hakodate	80.2	14.6	534	Arakawa	96.0	14.5	2059
Aomori	81.8	14.8	1089	Yonezawa	92.9	14.6	1250
Iwaki	82.8	14.0	1204	Niitsu	86.0	14.0	165
Hirosaki	87.4	15.7	380	Hukusima	93.9	13.6	207
Owani	86.5	15.4	207	Taira	115.0	24.0	76
Akita	89.8	15.6	163	Nakano	96.0	14.0	182
Morioka	89.0	16.0	982	Toyama	95.9	13.8	238
Omagari	96.1	12.5	72	Sinonoi	107.4	18.0	172
Tono	81.0	16.0	191	Tokyo	117.0	16.2	136
Mizusawa	86.0	15.0	194	Tsu	109.3	18.6	81
Sakata	93.7	12.1	231	Nisinomiya	101.3	17.7	208
Sinzyo	85.5	12.0	166	Okayama	116.4	16.0	64
Sendai	103.6	21.7	465	Koti	125.5	35.0	118
(Second generation)							
Sapporo	80.3	14.0	779	Haranomati	106.4	23.7	4299
Hakodate	74.3	15.9	5684	Taira	113.5	21.1	517
Hirosaki	78.4	13.6	1350	Nakano	86.9	14.8	716
Owani	87.9	14.8	2472	Toyama	99.9	22.2	4962
Akita	89.5	15.7	421	Sinonoi	101.6	19.2	411
Omagari	91.6	16.0	169	Takayama	93.5	19.7	2636
Tono	80.3	14.0	612	Tokyo	105.4	22.5	405
Mizusawa	86.3	15.4	458	Yonago	95.6	22.8	1648
Sakata	94.0	16.5	2691	Inatake	104.0	27.0	185
Atsumi	92.4	19.8	618	Tsu	117.2	22.8	396
Siogama	91.5	16.0	801	Nisinomiya	91.4	17.0	388
Sendai	100.8	18.8	4214	Okayama	133.9	22.7	305
Yamagata	91.7	19.5	6848	Taizi	116.0	19.0	183
Arakawa	92.8	17.5	2931	Hukuoka	113.0	22.9	5114
Yonezawa	82.7	16.7	4974	Koti	125.3	22.4	278
Niitsu	93.8	14.6	435	Miyazaki	111.3	23.5	458
(Third generation)							
Sapporo	75.6	10.5	777	Toyama	86.5	22.7	278
Hirosaki	74.9	14.4	307	Sinonoi	101.7	18.9	602
Akita	81.7	14.5	460	Tokyo	90.3	19.5	437
Tono	79.3	14.2	372	Inatake	103.7	17.9	703
Mizusawa	75.1	18.4	604	Tsu	98.3	22.9	630
Siogama	85.5	24.8	974	Okayama	112.5	21.1	692
Taira	105.2	26.4	2872	Taizi	113.1	22.5	768
Nakano	88.5	23.9	1975	Koti	108.1	20.4	490

\bar{x} = mean duration of the egg stage in days ; *s* = standard deviation ; *n* = number of nymphs that hatched.

observed in the successive tests at 20° and 25°C. As shown in the table, four strains out of the five that had been originated from Tokyo and farther south hatched on the average in about 100 days ; on the contrary five out of the six northern strains required less than 90 days. Among the latter the northernmost strain which had been derived from Sapporo completed development about one month earlier than did the most delayed strains from Tsu or Koti among the southern group.

At 15°C. The eggs of the Emma field cricket completed diapause at 15°C irrespective of the place of their origin. They began to develop rapidly when they were removed after a certain period and kept at a warm temperature. If eggs of some local strains were constantly kept at 15°C, they resumed post-diapause development subsequently to the completion of diapause. Nevertheless most of them failed to hatch under such conditions, and so this temperature was thought to be close to the lower

Table 4. *The durations of the incubation period at 30°C in different local strains of the Emma field cricket.*

Strain	\bar{x}	<i>s</i>	<i>n</i>	Strain	\bar{x}	<i>s</i>	<i>n</i>
Sapporo	76.0	11.6	146	Tokyo	100.1	17.3	95
Hirosaki	89.4	18.2	120	Tsu	107.1	20.0	62
Akita	84.4	17.9	36	Nisinomiya	83.0	15.8	119
Omagari	86.1	11.7	53	Okayama	99.8	14.5	32
Sinonoi	94.2	14.4	77	Koti	107.2	21.4	78
Toyama	85.9	17.3	181				

\bar{x} = the mean duration of the egg stage in days. *s* = standard deviation.
n = number of nymphs that hatched.

Table 5. *Percentage of embryos that had completed revolution during continuous exposure to 15°C for nine months in different local eggs of the Emma field cricket.*

Strain	Gen.	%	<i>n</i>	Strain	Gen.	%	<i>n</i>
Sapporo	II	83.0	200	Taira	I	2.0	200
Hirosaki	II	75.5	200	Nakano	I	45.0	200
Akita	II	32.0	200	Toyama	II	17.5	200
Tono	I	54.0	200	Tokyo	II	0	200
Mizusawa	I	32.0	200	Tsu	II	0	200
Sakata	II	28.5	200	Okayama	II	0	200
Niitsu	I	26.5	200	Koti	II	0	200

n = number of total eggs.

threshold for normal hatching. UMEYA (1946) also reported that the lower threshold for the post-diapause development was not far below 15°C in the Emma eggs of the Tokyo population.

At this critical temperature, the percentage of eggs which had resumed to develop within a period of nine months varied remarkably among local strains (table 5.) At the end of the observation period, most eggs coming from Hokkaido and northern Honsyu had passed through revolution, while none of those from the southern localities showed any sign of post-diapause development. About one-third of the eggs had undergone revolution in most strains from the central parts of the country.

It was not certain whether this local difference was due to different durations of diapause at this temperature or to different thresholds for the resumption of post-diapause development. The fact is that the northern eggs are more competent to develop at the low temperature than are the southern ones.

Cold exposure. Provided that eggs of different local strains were continuously kept at 5-10°C, none of them initiated post-diapause development. When they were transferred at a warm temperature, some of them hatched in a short period, the proportion doing so being dependent on the duration of previous cold exposure. This implies that diapause is broken but the embryonic development is not resumed at the cold temperature.

There was a little difference among the local eggs in the time required for hatching at a warm temperature after an adequate duration of cold exposure. In the northern strains, the peak of hatching appeared between the 16th and 17th days of the post-diapause incubation at 25°C while it occurred between the 19th and 22nd days in the southern strains. Different durations of post-diapause incubation should therefore be allowed for different strains in order to define unretarded hatching after cold exposure.

In each local stock the percentages of eggs hatching without delay were plotted

against the durations of previous cold exposure ; the curves thus obtained were usually sigmoid in shape. Transformation of the percentages into probits gave a straight line as graphically shown in previous papers (MASAKI, 1962, 1963). The median effective durations of cold exposure for breaking diapause were determined from this line and thereby the diapause intensity in different local strains were represented. As shown by the results in table 6, the median duration was about 50 days in the Sapporo or certain other northern strains, whereas it varied from two to three months among the central and southern strains. The southernmost eggs from Koti required about 100 days of chilling to become free of diapause.

Table 6. *The median effective duration of cold exposure (MED) at 5-10°C for the termination of diapause in different local eggs of the Emma field cricket.*

First generation		Second generation	
Strain	MED (days)	Strain	MED (days)
Sapporo	47	Sapporo	47
Hirosaki	57	Hirosaki	57
Akita	48	Akita	55
Omagari	51	Omagari	66
Tono	59	Sakata	55
Mizusawa	67	Toyama	69
Niitsu	69	Sinonoi	71
Taira	76	Tokyo	78
Nakano	61	Inatake	84
Sinonoi	69	Tsu	91
Tsu	78	Taizi	99
Koti	98	Koti	115

Although in certain local samples the intensity of diapause varied considerably between different generations, a consistent geographic gradient is perceived in the table. The tendency is substantially identical to that found at high temperatures. That is, the intensity of egg diapause decreases northward. Between the northernmost (Sapporo) and southernmost (Koti) strains there was a difference of about 50-60 days in the median length of the cold exposure required for unretarded development of the eggs. This is no less than the difference in the duration of the entire incubation period at 25°C between them. From this fact, it may be assumed that the local difference in the incubation period is mainly due to the variation in the intensity of diapause and that the rate of embryonic development is less variable.

The response of eggs to a still lower temperature of $0 \pm 1^\circ\text{C}$ was tested by a similar method, but the median effective duration of exposure for breaking diapause was non-existent or unable to be determined. The response of eggs was irregular in some cases or the number of hatch did not exceed 50 percent in others with any duration of the cold exposure. Such results were probably ascribed to an unfavourable effect of this cold temperature. HOGAN (1960b) reported that in *Teleogryllus commodus* all of the diapause eggs perished by one-month exposure to 0°C , but that shorter exposures were highly effective in breaking the diapause. The treatments for the Emma eggs lasted too long to visualize such a diapause-breaking action of 0°C ; the data indicate, however, that the Emma eggs are more resistant to cold than those of the Australian species. Certain proportions of the eggs survived the exposure for as long as three months or more and they hatched in a short period when incubated at 25°C.

Despite the irregular responses, the local strains were conspicuously different in

Table 7. *Maximum percentage hatch among series of batches of 100-150 eggs which were exposed to $-1-1^{\circ}\text{C}$ for periods ranging from 3 to 19 weeks in different local strains of the Emma field cricket.*

Strains	%	w	Strains	%	w
Sapporo	40	15	Nakano	37	15
Hirosaki	64	19	Sinonoi	23	13
Akita	50	15	Toyama	33	17
Omagari	14	15	Tokyo	15	17
Tono	73	15, 17	Inatake	23	13
Mizusawa	29	19	Tsu	2	11, 19
Sakata	66	15	Nisinomiya	12	13
Siogama	49	15	Okayama	3	19
Niitsu	49	15	Taizi	2	11
Taira	18	15	Koti	2	15, 17

w = duration of cold exposure in weeks for the maximum percentage hatch obtained.

their hatchability after exposure to $0 \pm 1^{\circ}\text{C}$. This is clearly indicated by the maximum percentage hatching observed in each local series of nine batches of 100 eggs exposed to the cold temperature for 3 to 19 weeks (table 7). A number of nymphs hatched from the northern eggs without delay, when transferred at a high temperature, in most cases after 15 weeks of the cold exposure but only a few from the southern eggs. Further prolongation of the cold treatment did not increase the hatchability. From this, it seems that the northern eggs are able to tolerate better the cold temperature than are the southern eggs. Consequently, the former are better adapted to their colder native environment.

After hibernation. The Emma eggs require only a few days for pre-diapause development and about two to three weeks for post-diapause development at 25°C (MASAKI, 1960). They spend most of their time in diapause. It is likely that the variation in the duration of the incubation period is mainly due to the different intensity of diapause among local populations. The results of the experiments described above support this view, but it was also observed that the hatching time after cold exposure was not uniform among different local eggs.

In order to confirm this observation, egg samples laid by twenty local stocks of mixed generations in the autumn of 1962 were kept in an unheated cellar during the winter months, and in the following spring they were incubated at a temperature of 24°C . The numbers of hatching nymphs were recorded daily until the peak of hatching became apparent and the duration of the incubation period was represented by the mode. The results are summarized in table 8. As shown in the table, the northern strains hatched earlier than the southern ones, though the difference between the extremes was only less than one week. The difference of this sort would be more conspicuous if the eggs were incubated at a lower temperature.

It was not certain whether this variation was due to the variable rate of post-diapause embryonic development or to the variable amount of residual diapause. In any case the great variation in the intrinsic incubation period was very much reduced during the course of hibernation. This leads to a conjecture that the variation largely concerns with adaptation to the local environment during the first half of the overwintering period. However, the variation is still detectable in the post-hibernation period and the adaptive meaning of this should not be ignored. There is a close correlation between the duration of post-hibernation incubation and the latitude of the source of the eggs ($r = -0.897$, $n = 23$). The hatching date in various areas would not be ex-

Table 8. *The modes of hatching time in days at 24°C in local strains of the Emma field cricket after hibernation in a cellar.*

Strains	<i>M</i>	<i>n</i>	Strains	<i>M</i>	<i>n</i>
Hakodate	18	387	Taira	22	264
Iwaki	18	589	Toyama	20	479
Hirosaki	19	366	Takayama	19	1156
Owani	18	353	Tokyo	22	635
Atsumi	18	737	Yonago	22	165
Sendai	19	1025	Taizi	24	200
Yamagata	19	292	Hukuoka	20	177
Arakawa	19	828	Miyazaki	24	274
Yonezawa	19	203	Hukiagehama*	24	205
Haranomati	20	345	Tanegasima**	24	233

M = mode of hatching time. *n* = number of hatching nymphs.

* 31.52°N. ** 30.43°N.

plicable by the local temperature accumulation alone.

ANALYSIS

Reaction norm. In poikilothermal animals, there may be several different ways in which a variation occurs in the duration of developmental stages at a given temperature. Individuals or strains may vary in the intrinsic duration of development, though they are similar in the norm of response to temperature. Also, they may be different in the temperature coefficient for development or in the norm of response to temperature. The latter situation has been known in the frog, *Rana pipiens*, in North America (MOORE, 1949). In this animal the geographic variation in the temperature response is so remarkable that the northern eggs develop more rapidly than the southern ones at low temperatures but the trend is reversed at high temperatures. It is also possible that local populations are differentiated in both the reaction norm and the duration of development. *Austroicetes cruciata* affords such an example. The eggs of this grasshopper from southern and western parts of Australia differ from one another not only in the optimum temperature but also in the duration of diapause development under the conditions optimum for each local population (ANDREWARTHA, 1952).

From this point of view, it seems necessary to envisage the data thus far described for the Emma field cricket. In doing so, appropriate measurements which represent the local features of the eggs could be chosen to visualize the general picture of the geographic varia-

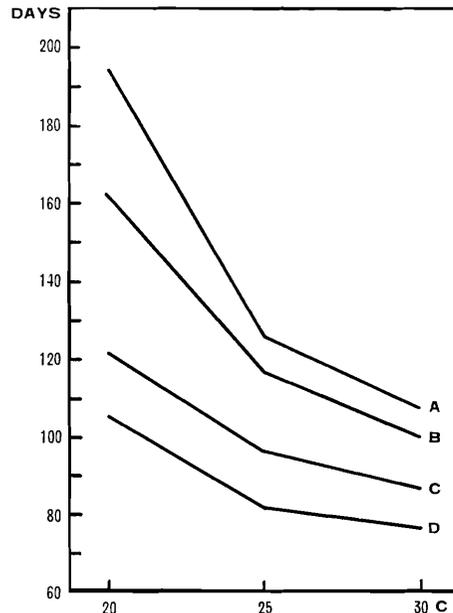


Fig. 3. Relation between the duration of the diapause egg stage (ordinate) and temperature (abscissa) in four local strains of the Emma field cricket. A, Koti, 33.6°N. B, Tokyo, 35.7°N. C, Toyama, 36.7°N. D, Sapporo, 43.1°N.

tion. Thus in figure 3 are given the relationships between the duration of the egg stage and temperature in four local strains which had been originated from the northern, central and southern areas of this archipelago. The curves in this figure are almost parallel with each other, which means that there are no remarkable qualitative differences in the mode of response to temperature among the local strains. The incubation period is shortened by raising temperature and lengthened by lowering it in a similar way in these local samples. Comparisons among the data in tables 2, 3 and 4 show that the response is alike in many other strains. A strain which hatched earlier at a high temperature usually did so at a lower temperature as well, so that the relative difference between strains remained almost constant within the range between 20° and 30°C. A similar situation persists in lower ranges of temperature. At a critical temperature of 15°C more eggs developed in a given period of time in those strains which took less time to hatch at a high temperature. Again the strains with a relatively short incubation period at a warm temperature took less time for terminating diapause at a cold temperature. The local difference is therefore mainly in the intrinsic *duration* of the incubation period.

Nevertheless there is probably a slight local variation in the mode of response to temperature. The curves in figure 3 differ not only in height but also slightly in slope so that the southern eggs are more sensitive to the retarding effect of low temperatures than are the northern eggs. Local differences in the developmental threshold may be partly responsible for the different competence of the local eggs to develop at 15°C. Also, the experiments on the hatchability after exposure to $0 \pm 1^\circ\text{C}$ suggest that the local strains differ in their lower limits of tolerance to temperature. There may be still other subtle local variations, but in the present data there are close correlations between the responses of eggs to different conditions of temperature (table 9). The characteristics of the local eggs may therefore be represented by the duration of the incubation period at any temperature, at least for the purpose to pursue the broad geographic tendency.

Table 9. *Correlations between responses of eggs to different temperature conditions in local strains of the Emma field cricket.*

Response	Temp.	Gen.	Mean duration of egg stage at :			
			20°C		25°C	
			<i>r</i>	<i>n</i>	<i>r</i>	<i>n</i>
Mean duration of egg stage	30°C	I	+0.829	11	+0.865	11
Mean duration of egg stage	20	I			+0.778	21
		II			+0.874	14
		III			+0.826	11
Percentage of developing eggs MED for diapause termination	15	I, II	-0.786	13	-0.843	14
	5-10	I	+0.907	12	+0.803	12
Hatchability after exposure	-1-1	II	+0.990	7	+0.943	12
		I, II	-0.734	17	-0.817	20

r = correlation coefficient. *n* = number of pairs.

Heritability. The data given above, especially those at 20° and 25°C at which the results with successive generations were available, provide information on the heritability of the local features of cricket eggs. It seems that the intrinsic *duration* of incubation

at a given temperature is largely determined by genetic factors, even though it was subject to a large fluctuation from one generation to the next. The persistence of a similar geographic tendency through the successive generations in the experiments at 20° or 25°C, as well as in the cold exposure tests, is difficult to interpret were it not based on the genetic differences among the local populations. The heritability of the local traits is clearly indicated by the scatter diagrams in figures 4 and 5, in which the mean incubation periods of the progeny are plotted against those of the parent or grandparent. Although only a small number of local strains were continuously available, there were found close correlations between the mean incubation periods of the parent and progeny both at 20° and 25°C. The correlation coefficients obtained at the former temperature are 0.946, 0.938 and 0.900 between the first and second, first and third, or second and third generations, respectively. The respective values at 25°C are 0.895, 0.907 and 0.923. These results are in agreement with a previous conclusion reached by intercrossing the northern and southern strains (MASAKI, 1963).

The regression of the mean incubation period of the progeny on that of the parent or grandparent is linear in every case, but the regression coefficient is variable. It ranges from 0.71 to 1.02 at 25°C and from 0.64 to 1.02 at 20°C, depending on generations arranged in pairs. The coefficients for the regression of the second on the first generation are very close to unity at both temperatures, but the smaller values are obtained for the regression of the third on either the second or the first generation. As stated before, a considerable number of strains completed their incubation in somewhat shorter periods of time in the last than in the preceding generations. This is apparently responsible for such deviations of the regression coefficient, which is theo-

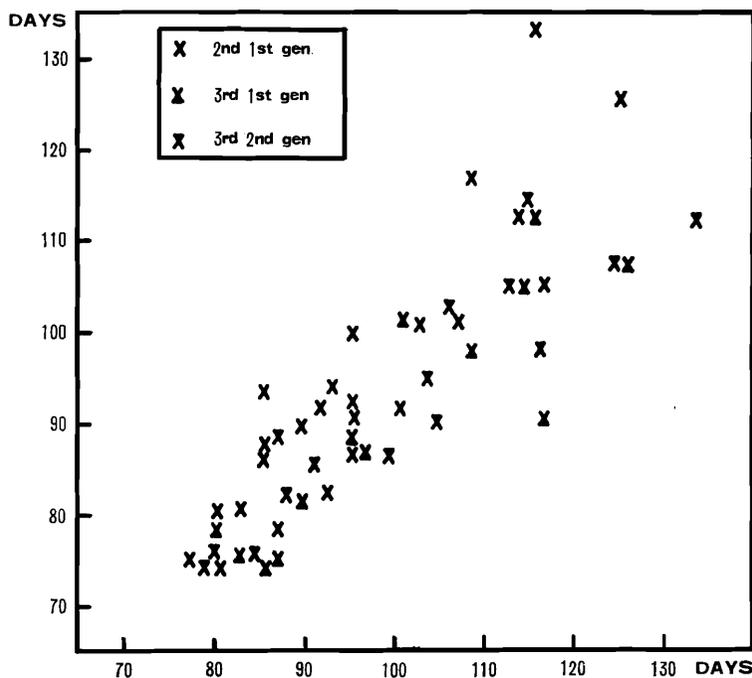


Fig. 4. Correlations among the mean durations of the egg stage at 25°C of different generations in local strains of the Emma field cricket.

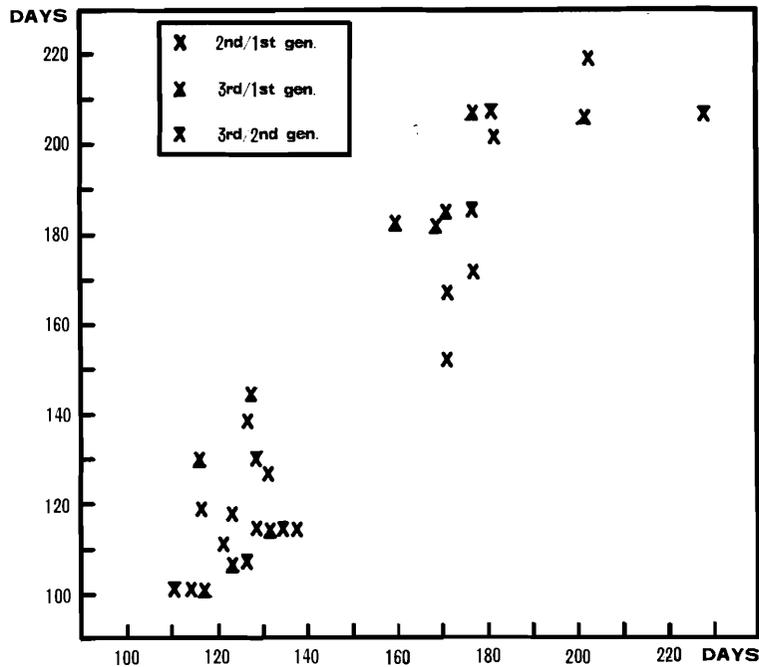


Fig. 5. Correlations among the mean durations of the egg stage at 20°C of different generations in local strains of the Emma field cricket.

retically unity if all errors were excluded. At any rate there is no conclusive evidence in the present data to deny the heritability of the local characteristics of the eggs of the Emma field cricket.

Geographic trend. From the lot of data hitherto presented, it is already apparent that the characteristics of the eggs are more or less closely correlated with the geographic location of their native habitats. In the present section a more quantitative approach will be attempted to visualize the pattern of this geographic variation.

Since the geographic location of each strain is designated by the three variables, latitude, longitude and altitude, their correlations with the response of eggs observed in different conditions of temperature were calculated at first. The results are arranged in table 10. The length of the incubation period at three constant temperatures and the

Table 10. Correlations between the responses of local eggs and the latitude (X_1), longitude (X_2) or altitude (X_3) of the native habitat in the Emma field cricket.

Response	Temp.	Gen.	X_1	X_2	X_3	n
Mean duration of egg stage at :	30°C	I	-0.693	-0.594	+0.110	12
		I	-0.842	-0.680	-0.201	28
	20	II	-0.782	-0.633	-0.190	32
		III	-0.851	-0.778	-0.057	16
		I	-0.659	-0.441	-0.256	21
		II	-0.768	-0.612	-0.249	14
Percentage of developing eggs at :	15	III	-0.848	-0.794	-0.151	11
		I, II	+0.884	+0.612	+0.363	14
		I, II	-0.790	-0.728	-0.077	31
MED for diapause termination at :	5-10	I, II	+0.742	+0.745	+0.186	19
Hatchability after exposure to :	-1-1	I, II				

n = number of pairs.

duration of chilling for breaking diapause are correlated negatively with latitude or longitude. The percentages of developing eggs at 15°C and of maximum hatch after $0 \pm 1^\circ\text{C}$ exposures are positively associated with the same geographic variables. For all the measurements, the degrees of association are consistently higher with latitude than with longitude. None of the eggs' responses show close correlations with altitude, though the correlation coefficients consistently take negative values except for the mean incubation period at 30°C.

The existence of close correlations with latitude and longitude justifies to state that, in the inherited duration of the egg stage of the Emma field cricket, there is a geographic gradient from the northeastern to the southwestern extremities of this country. The whole picture of this clinal variation is illustrated in figures 6 and 7, in which are plotted the mean incubation periods of the local strains at 20° or 25°C, respectively, against the latitude of the sample source.

The geographic regularity of the variation strongly suggests that climatic factors which vary with latitude have exerted selection pressure upon the developmental feature of the local eggs. The rather high correlations with longitude may be merely a manifestation of the geographic position of the archipelago which extends from northeast to southwest so that latitude varies with longitude as one travels through this country. On the other hand, the small correlations with altitude may not necessarily exclude the significance of this variable. In our local samples, altitude varies independently with latitude ($r = 0.03 - -0.11$); therefore, the strong association of the eggs' hereditary character with the latter may obscure its correlation with the former.

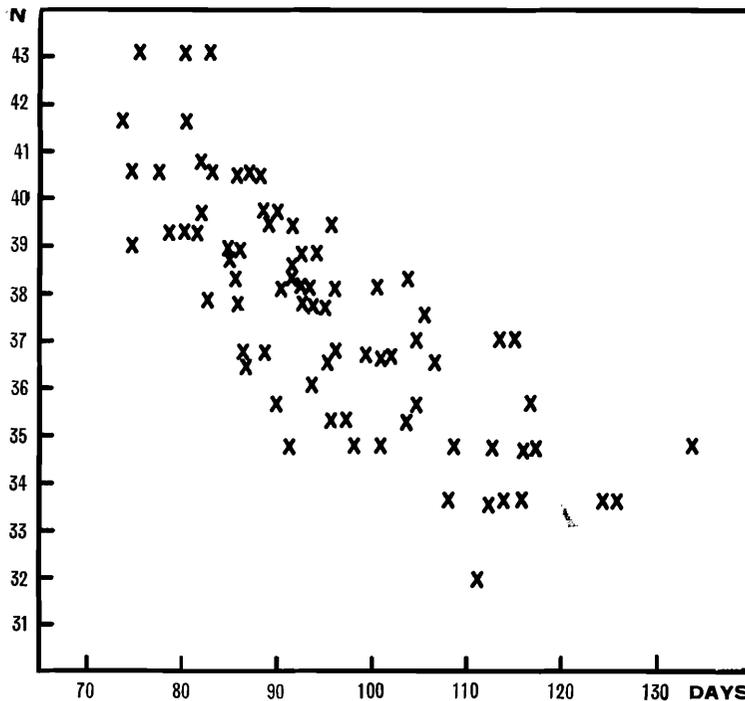


Fig. 6. The intrinsic durations of the egg stage at 25°C (abscissa) in different strains of the Emma field cricket in relation to the latitude of their native localities (ordinate).

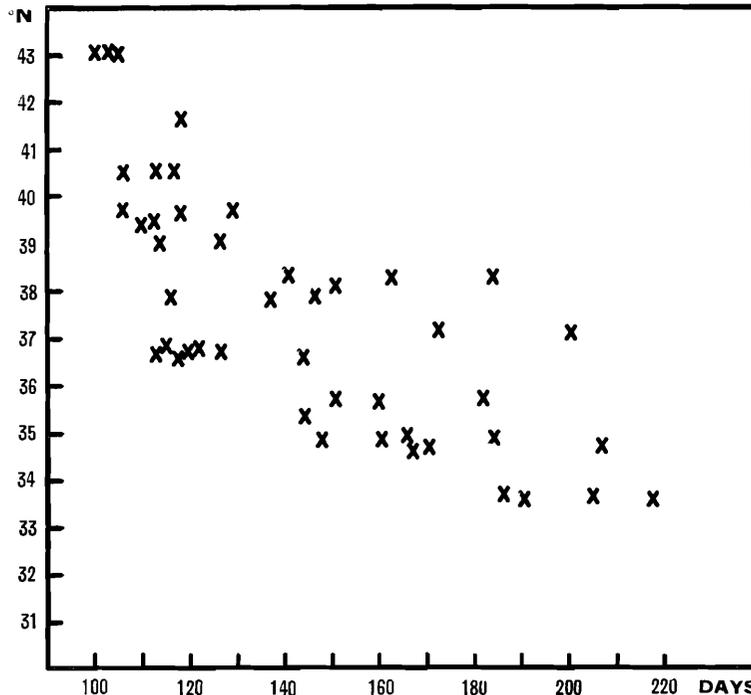


Fig. 7. The intrinsic durations of the egg stage at 20°C (abscissa) in different strains of the Emma field cricket in relation to the latitude of their native localities (ordinate).

This difficulty is eliminated by means of multiple regression analysis, in which each effect of the three geographic variables are evaluated independently of the others (SNEDECORE, 1956). Only the mean incubation periods at 20° and 25°C were subjected to this analysis, just because the data available are relatively extensive. Moreover, the discussion on the norm of response has led to the conclusion that the geographic tendency might be represented by the incubation periods of local eggs at these temperatures. The computation was carried out on the variation common for the three successive generations at each temperature in order to obtain the average regression. The partial regression coefficients for the mean incubation period on latitude, longitude or altitude were obtained at first, whereby the variance among the local means was analysed. The results are summarized in table 11, in which the multiple regression equations predicting the mean durations of the local egg stage at 20° or 25°C are also given.

The partial regression coefficient on latitude (X_1) as well as on altitude (X_3) takes a negative value, but it is positive on longitude (X_2) consistently at both temperatures. This implies that the negative simple correlation of longitude with the local incubation period has no real significance. On the contrary, the analysis suggests a tendency that the incubation period varies proportionally with longitude. The egg stage thus lengthens eastward if local samples from the same latitude and altitude are compared. The small variance ratio for longitude shows, however, that such a tendency could not be considered as significant. From the analysis of variance, it is further elucidated that the multiple regression on X_1 , X_2 , X_3 accounts for about 66-70 percent of the local varia-

Table 11. *Analysis of variance for the mean duration of the diapausing egg stage in local strains of the Emma field cricket by means of the multiple regression on latitude, longitude and altitude.*

Source of variation	For Y at 25°C				For Y at 20°C			
	f	Sum of squares	Mean square	F	f	Sum of squares	Mean square	F
Total	73	12791			43	47606		
X_1, X_2, X_3	3	8906			3	31704		
Deviation	70	3885	56		40	15902	398	
X_1, X_2	2	8531			2	27089		
X_1, X_3	2	8889			2	30890		
X_2, X_3	2	5863			2	18387		
X_1 after X_2, X_3	1	3043	3043	54.3**	1	13317	13317	33.4**
X_2 after X_1, X_3	1	17	17	0.2	1	813	813	2.0
X_3 after X_1, X_2	1	374	374	6.7*	1	4615	4615	11.6**
Regression equation		$\hat{Y}=226-4.645X_1+0.334X_2-0.016X_3$				$\hat{Y}=163-12.076X_1+3.178X_2-0.084X_3$		
		$\hat{Y}=261-4.351X_1-0.015X_3$				$\hat{Y}=510-9.591X_1-0.073X_3$		
		$\hat{Y}=259-4.341X_1$				$\hat{Y}=500-9.492X_1$		

Y = the mean duration of egg stage at the constant temperatures.

X_1 = latitude. X_2 = longitude. X_3 = altitude.

f = degrees of freedom. F = variance ratio.

* significant at $P = 0.05$. ** significant at $P = 0.01$.

tion in the intrinsic incubation period, and also that latitude contributes to the major part of the regression. The regression due to altitude is also significant, though it takes a far smaller part of the total variation as compared with latitude. The deletion of the effects of latitude and altitude from the variation due to the entire regression leaves no significant portion for the effect of longitude as already mentioned. A multiple regression equation on latitude and altitude would therefore suffice to describe the geographic trend of the variation. The equation given at the bottom of table 11 suggests that the mean intrinsic duration of the incubation period at 25°C decreases about 3 to 4 days as the geographic location of the habitat shifts one degree of latitude northward as well as 200 meters higher above the sea level. The intrinsic decreases in the incubation period at 20°C for the same shifts of the habitat in latitude and altitude are about 9 and 14 days, respectively. Allowing very rough approximations, a horizontal shift of one degree latitude and a vertical shift of 200 m in the location of habitat are comparable in their effects on the intrinsic variation of the incubation period among the local populations. This is suggestive, for in these parts of the range the annual mean temperature changes at a lapse rate of about 1°C for each one degree of latitude as well as for each 200 m of altitude. Hence it seems to be some climatic factor which is directly responsible for the hereditary variation.

Analysis of variance for the data with each generation leads to a similar conclusion except for the lack of significance of the partial regression on altitude. In each generation the regression on latitude occupies the greatest proportion of the total variation and that on longitude is not significant at all.

The conclusion to be drawn from these analyses is evident : in the hereditary duration of the incubation period of the Emma field cricket, there occurs a clinal variation which extends from about 31° to 43°N in this eastern periphery of its range ; the intrinsic egg stage thus increases from north to south and on this major latitudinal trend is superimposed a variation relating to altitude.

Climatic relations. It seems quite probable from the above analyses that the clinal variation has been established by climatic selection. Therefore, the correlations of climatic conditions with the incubation periods of the local samples at 20° or 25°C are to be investigated next. Several climatic indices were selected for this purpose by the reasons stated below.

Among various climatic factors temperature might be most important, because it is not only of vital importance for the development and survival of the eggs but also most closely correlated with both latitude and altitude. The general conditions of temperature at each locality would be represented simply by the annual mean temperature (X_4). But two localities with similar annual mean temperatures may differ in hotness or coldness. It is necessary to separately estimate the relations of the summer and winter conditions. To this end, two indices were chosen: a warmth index (X_5) which sums [(monthly mean temperatures higher than 15°C) - 15°C], and a coldness index (X_6) which sums [15°C - (monthly mean temperatures lower than 15°C)]. These are modifications originally invented by KIRA (1945) in that 15°C is taken, instead of 5°C, as a threshold temperature.

It may well be that the conditions to which the cricket eggs are directly exposed are more important than those experienced at the other stages in the life cycle. Still other indices are thus utilized to represent the features of climatic conditions during the overwintering period: the sum of the monthly means of daily maximum temperature from October to April (X_7), and the sum of the monthly means of daily minimum temperature during the same period (X_8). The adults of this cricket are at their peak of breeding activity in the latter half of September. Owing to the adaptive variation in the rate of nymphal development, there are no remarkable local differences in the seasonal appearance of the adults. In almost everywhere the majority of eggs are probably laid before the end of October. Although their hatching dates after hibernation are unknown, nymphs do not appear at least before the middle of May. At Hirosaki, very small nymphs probably in the first or second instars are occasionally found at the end

Table 12. *Correlations between the duration of the diapausing egg stage at 20° or 25°C and several climatic variables in the local strains of the Emma field cricket.*

Temp.	25°C			20°C		
	I	II	III	I	II	III
Generation						
X_4	+0.783	+0.774	+0.858	+0.642	+0.826	+0.860
X_5	+0.752	+0.724	+0.846	+0.531	+0.717	+0.880
X_6	-0.829	-0.790	-0.850	-0.679	-0.853	-0.842
X_7	+0.905	+0.825	+0.866	+0.745	+0.909	+0.895
X_8	+0.744	+0.706	+0.762	+0.661	+0.792	+0.778
X_9	-0.862	-0.807	-0.787	-0.775	-0.864	-0.950
X_{10}	+0.271	+0.368	+0.314	+0.086	+0.368	+0.217
n	19-26	22-31	11-16	15-21	10-14	9-11

X_4 = annual mean temperature.

X_5 = sum of [(monthly mean temperature above 15°C) - 15°C].

X_6 = sum of [15°C - (monthly mean temperature below 15°C)].

X_7 = sum of monthly means of daily maximum temperature from October to April

X_8 = sum of monthly means of daily minimum temperature from October to April.

X_9 = number of days with snow cover.

X_{10} = annual precipitation.

n = number of pairs; the number varies because certain climatic data were not available in certain localities.

of May. Insectary rearing of this cricket at Tsu suggests as well that the hibernated eggs do not hatch until late spring or early summer even in this warmer locality. The hibernating period was therefore tentatively considered as lasting from October to April.

Another factor of importance during this period is the number of days with snow cover (X_9), because the eggs, being deposited in the soil, are protected from violent fluctuations of temperature when the ground is covered with snow. The annual amount of precipitation (X_{10}) appears to be of little ecological significance, because moisture would scarcely be a limiting factor under the rather moist climates of this region, though it has been known that contact moisture is essential for cricket eggs (BROWNING, 1953 ; MCFARLANE *et al.*, 1959 ; MASAKI, 1960).

The correlation coefficients between the mean incubation periods and the climatic indices are given in table 12. The coldness index and the number of days with snow cover correlates negatively and all others positively with the intrinsic duration of the incubation period. The coefficients are all similarly large except for annual precipitation. Comparisons between coefficients in each generation series reveal, however, that there is a rather consistent order among the climatic indices in respect to their degrees of association with the trait of the eggs. The closeness of correlation becomes smaller in the following order : X_7 , X_9 , X_4 , X_5 , X_8 and X_{10} . It is interesting that the daily maximum temperature during the hibernating period (X_7) ranks first in the above order while the daily minimum during the same period (X_8) comes next to the tail end. If a biological meaning is to be sought for this fact, it may be that the maximum daily temperature is more hazardous than the minimum for the survival of hibernating eggs. The latter is consistently below the threshold for post-diapause development, but the former may sometimes exceed it during the hibernation period. If the eggs are untimely reactivated by warmth before or during the winter months, they would certainly be killed by frost. If they are not reactivated, they remain to be resistant to cold. Hence, the intensity of diapause, and therefore the intrinsic duration of incubation, would be selected more rigorously by the maximum than by the minimum daily temperature.

When the simple regression of the hereditary hatching time on each of these climatic indices is considered, the square of each correlation coefficient measures the fraction of the variation attributable to the regression. The regression on the daily maximum temperature during the hibernation period accounts for 56-83 and 68-82 percent, and that on the number of days with snow cover for 60-90 and 62-74 percent, of the local variations measured at 20° and 25°C, respectively. The other variables are more or less inferior in their predicting powers, and annual precipitation practically contributes nothing to the local variation of the incubation period ; it explains only 1-14 and 7-10 per cent of the total variations at 20° and 25°C, respectively. Multiple regression analysis should be undertaken in order to infer more precisely the influence of each climatic factor independent of the others. There are, however, very close correlations among certain climatic indices themselves as represented by large correlation coefficients ($r = 0.90 - 0.98$). Under such circumstances the analysis would not yield fruitful results so that extensive computation was not undertaken. An attempt was made only to discriminate the relations of the climatic indices of the hibernating period. Unfortunately, the number of days with snow cover (X_9) correlates most closely with the daily maximum temperature, and the former was arbitrarily excluded from the analysis.

Thus table 13 gives the results of multiple regression analysis in which only two climatic variables, the daily maximum (X_7) and minimum (X_8) temperatures during

Table 13. *Analyses of variance for the mean duration of the diapausing egg stage in local strains of the Emma field cricket by means of the multiple regression on geographic and climatic variables.*

Source of variation	For Y at 25°C				For Y at 20°C			
	<i>f</i>	Sum of squares	Mean square	<i>F</i>	<i>f</i>	Sum of squares	Mean square	<i>F</i>
Total	70	12437			43	47606		
X_7, X_8	2	9217			2	34123		
Deviation	68	3220	47		41	13483	329	
X_7 alone	1	9085			1	33682		
X_8 alone	1	6588			1	26813		
X_7 after X_8	1	2629	2629	55.9**	1	7310	7310	22.2**
X_8 after X_7	1	133	133	2.8	1	441	441	1.3
Regression equation		$\hat{Y} = 45.92 + 0.693X_7 - 0.163X_8$				$\hat{Y} = 23.88 + 1.647X_7 - 0.411X_8$		
		$\hat{Y} = 54.84 + 0.553X_7$				$\hat{Y} = 46.53 + 1.293X_7$		
X_1, X_7	2	9283			2	33859		
Deviation	68	3153	46		41	13747	335	
X_1 alone	1	8680			1	27037		
X_7 alone	1	9085			1	33682		
X_1 after X_7	1	199	199	4.3*	1	177	177	0.5
X_7 after X_1	1	603	603	13.2**	1	6822	6822	20.2**
Regression equation		$\hat{Y} = 133.3 - 1.723X_1 + 0.362X_7$				$\hat{Y} = -46.04 + 2.013X_1 + 1.519X_7$		
		$\hat{Y} = 54.84 + 0.553X_7$				$\hat{Y} = 46.53 + 1.293X_7$		

Y = the mean duration of the egg stage at the constant temperature.

X_7 = sum of monthly mean of daily maximum temperature from October to April.

X_8 = sum of monthly mean of daily minimum temperature from October to April.

X_1 = latitude. f = degrees of freedom. F = variance ratio.

* significant at $P = 0.05$. ** significant at $P = 0.01$.

the hibernation period are taken into account. Most part of the total variation among the incubation period of local strains at 25° and 20°C is due to the partial regression on X_7 , the deletion of which leaves no significant part of the variation for X_8 . Despite the insignificant effect of X_8 , the partial regression coefficients on the two variables seem to offer interesting information. At both temperatures the coefficient on X_7 takes a positive value while that on X_8 is consistently negative. The implication of this may be as follows.

If the daily minimum temperatures are similar in different local habitats, there is a tendency that the intrinsic intensity of diapause would be stronger where the daytime temperature attains a higher maximum level. On the other hand, the daily maximum being equal, the intensity would be weaker where the night cold is less severe. If the risk of overgrowth induced by the daytime warmth remains the same, the mortality caused by night frost would be related to the severity of the climate, hence a stronger diapause would be favoured where the temperature drops to a lower minimum level. Biologically thinking, this would seem to be a possible interpretation. There is, however, no reasonable basis for such a speculation from the statistical point of view and the present data may not be suitable for such an elaborate analysis. It should be noted also that X_7 is very closely inversely correlated with the number of days with snow cover ($r = -0.95 - -0.98$). In the snowy parts of this country, the eggs are surely seldom exposed to the diurnal fluctuation in temperature during the coldest season. Under such circumstances the significance of temperature as a selecting agency would be more limited than it is visualized above.

It seems fair merely to state that the intrinsic length of the incubation period of the Emma field cricket is closely correlated to some component parts of the climatic conditions of the local habitat. One further point should be investigated, however, before closing this analysis even by such vague words. The possibility, though it seems unlikely, is as yet not excluded that the geographic variation merely reflects a pattern of spatial dispersion of certain genes from one to the other extremities of the range that has taken place without any adaptive meaning. The variation, if so, would be a function of the distance from a certain place. Under such circumstances, the geographic location of the habitat would be correlated more closely with the physiological trait of the eggs than is any climatic variable. This is tested in the lower half of table 13, where the analysis of variance for the mean incubation period was carried out by means of multiple regression on latitude (X_1) and the daily maximum temperature in the hibernating period (X_7). The two variables are chosen because the simple regression on each is highly significant. Each of them therefore accounts for most part of the total variation due to the multiple regression. Apparently, however, the effect of the climatic variable is much more significant than that of the latitude. If the effect of X_7 is deleted, there leaves only a small amount of variation ascribable to X_1 . Yet the variance ratio slightly exceeds the significant level for the data at 25°C, while it does not at all for those at 20°C. On the contrary, the effect of X_7 is highly significant after the deletion of the effect of X_1 at both temperatures. Thus, the regression on latitude loses its significance if a climatic variable is taken into account. The slight significancy left for the

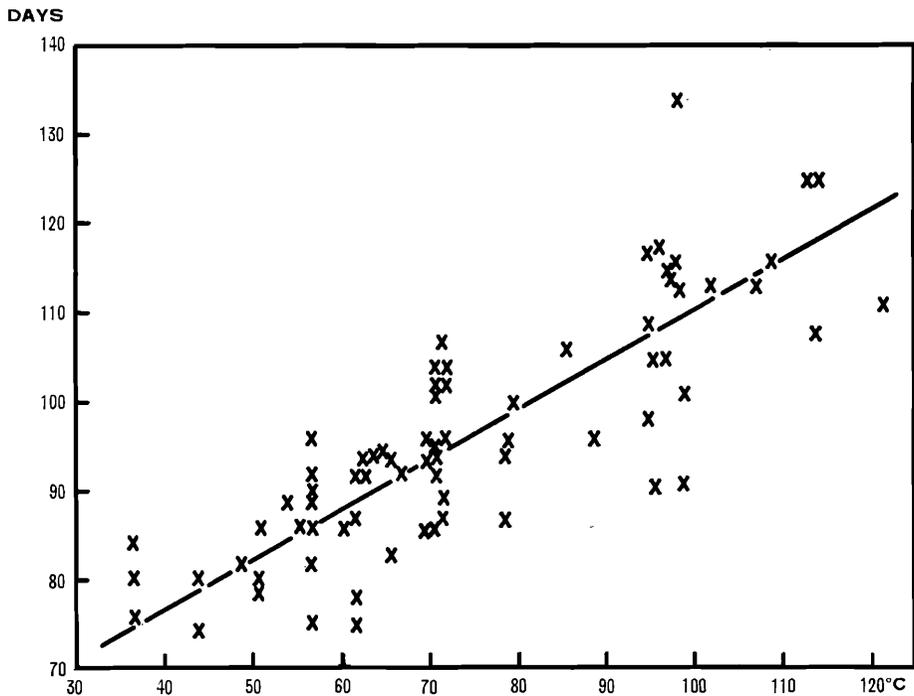


Fig. 8. Regression of the mean duration of the egg stage at 25°C (ordinate) on the sum of the monthly means of daily maximum temperature during the hibernation period in the native locality.

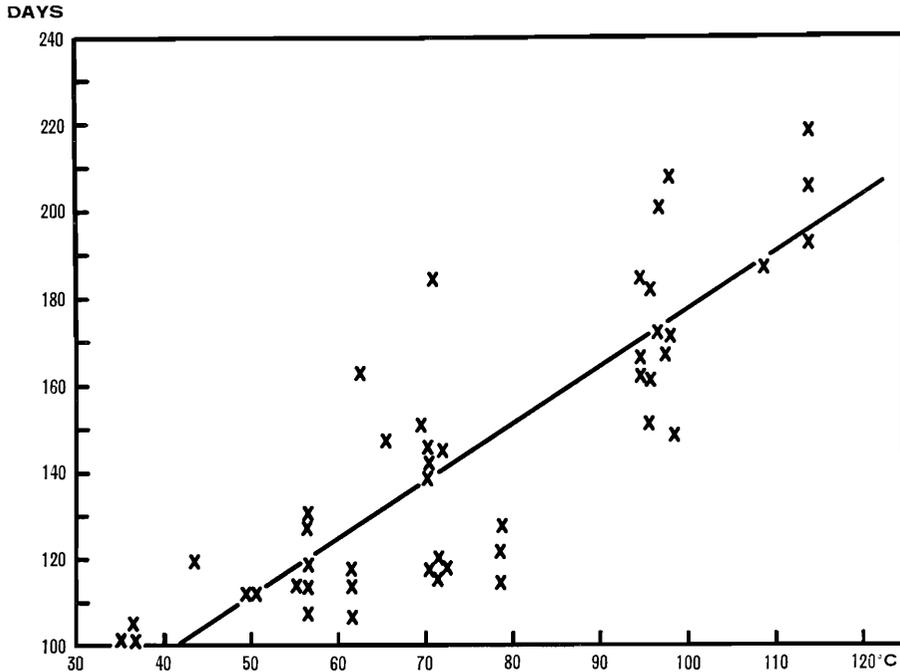


Fig. 9. Regression of the mean duration of the egg stage at 20°C (ordinate) on the sum of the monthly means of daily maximum temperature during the hibernation period in the native locality.

effect of latitude may be due to the effect of a factor other than X_7 that is correlated with latitude.

After all, the simple regression on the daily maximum temperature during the overwintering period describes sufficiently the pattern of the geographic variation in the intrinsic duration of the incubation period (figures 8 and 9). The regression on this variable alone accounts for more than 70 percent of the total geographic variation while even if all the three non-climatic variables (X_1 , X_2 and X_3) are combined they fail to explain the same proportion.

DISCUSSION

The polygenic control of diapause has been suggested in several species of insects (LE BERRE, 1951, 1953, 1959; SLIFER and KING, 1961; DANILEVSKY, 1957b; NAGATOMO, 1953; HARVEY, 1957). In the Emma field cricket, crossing experiments between the northern and southern strains show that the intensity of diapause is mainly controlled by chromosomal factors rather than by the maternal cytoplasm. No evidence has been obtained to suggest the action of a pair of alleles with simple dominant-recessive relations, for the reciprocal hybrids between the local strains are precisely intermediate in their diapause intensity (MASAKI, 1963). As given in the present work, there are continuous geographic shifts in this physiological trait. No clear-cut line of demarcation could be drawn in the clinal variation so that the character is presumably controlled by a polygenic system.

The intrinsic duration of the incubation period thus gradually increases southward in parallel with the climatic gradient. Yet there is no decisive evidence for the adaptive importance of the variation. In this study, the local eggs were kept under artificial conditions. Their responses thus observed are probably closely related to, but not at all the same as, their responses to the natural conditions of environment, the latter being more complex and changeable. Moreover, the cricket eggs have a phenotypic ability to adjust the intensity of diapause. The higher the temperature at the beginning of their embryonic life, the more intense would be the subsequent intensity of diapause (MASAKI, 1962). Consequently, diapause is more intensified towards the warmer south. The phenotypic response thus simulates the geographic variation — a situation often referred to as the BALDWIN effect (HUXLEY, 1942 ; MAYR, 1963). What is really important for survival is the response under the field conditions. The best way to test the fitness is perhaps to transplant the southern population to the north and vice versa, as carried out by PREBBLE (1941) with *Gilpinia polytoma* or by DANILEVSKY (1957a) with *Acronycta rumicis*. Comparisons between the life tables and the rates of reproduction of the immigrant and native populations should yield informative results. At present even such an unlikely interpretation cannot be ruled out that the intensity of diapause is merely determined by pleiotropic effects of genes responsible for other adaptive characters such as the developmental rate or cold-hardiness and that it has no survival value of its own. Although these questions still remain to be answered, the geographic tendency seems to suggest the way in which selection pressure exerts its effect upon the hereditary duration of the incubation period, or the intensity of diapause.

There are perhaps no external factors, other than temperature, that show regular geographic gradients so closely correlated with the variation. Temperature is therefore the most probable agency of natural selection. The geographic trend of the variation is just the reverse of the local duration of the cold season. The adaptation is doubtless not to the duration or severity of the local winter, but to the amount of effective temperatures. That is to say, a stronger diapause is at an advantage in the south, because of its greater ability to prevent untimely development when there is excessive warmth after the eggs have reached the cold-hardy stage. On the other hand, a weaker diapause suffices for the same purpose in the north (see MASAKI, 1961 for general discussions). Moreover, the cool environment probably favours a brief diapause, because the short growing season would be more important as a limiting factor for survival. The northern population would fail to complete development if their diapause lasts too long and delays the resumption of their development in the spring.

The variability in the developmental rate of the nymph is incorporated in this local adaptation in such a way that the seasonal life cycle is kept almost stable in different climates (MASAKI, 1963). The Emma field cricket achieves, so to say, a kind of phenological homeostasis through the genotypic response to climatic selection. As a consequence, its life cycle is well fitted to such habitats where a warm season alternates with a cold one. Namely, the adaptation of this species is definitely to temperate climates.

Although the Emma field cricket extends into the chain of small islands in the southwestern corner of this archipelago, it becomes less abundant rather abruptly towards the southern tip of Kyusyu. On the island of Tanegasima, off the southern coast of Kyusyu, this cricket is found only scarcely. The offspring of a few specimens, which were collected from this warm island, obligatorily entered diapause in the egg stage, undergoing a univoltine life-cycle. This persistence of univoltinism is impressive, when

the climatic conditions of this island is compared with those of the northernmost habitats. In the southern island, temperatures higher than 15°C prevail during eight months from April to November, while at Sapporo, Hokkaido, during only four months from June to September. The temperature accumulation above 15°C, roughly estimated from the monthly means, is about 1,763 day-degrees in the former island, and is almost four times as great as in the latter where it attains only about 435 day-degrees. The cricket could therefore produce a number of generations a year in the warmer environment were its diapause facultative or non-existent in the south and its development as rapid as in the north. Although the vast areas of continental Asia are not explored where the situation is quite unknown, there is as yet no evidence of geographic variability in the voltinism of this cricket. As long as the adaptability is restricted within the framework of the univoltine life cycle, a stronger diapause and slower development would be favoured in the warmer environment.

If the situation stated above occurs also in the other parts of the species range, it seems probable that the species cannot extend farther south into tropical countries. This is due to the disadvantage of the firm diapause in the region where warm or hot weathers prevail all the year round. BIGELOW (1960b) observed that rearing at constant high temperatures was deleterious for *Gryllus pennsylvanicus* and *Gryllus veleties*, both of which enter obligatory diapause. He stated that the evolution of cold-hardiness has apparently involved a reduction in the tolerance to constantly warm environment. Whether or not the same is true for the Japanese species of *Teleogryllus* is as yet not certain, but it is at least obvious that an obligatory diapause does nothing more than retarding and disturbing the maturation rate where there is no cold season.

In this connexion, it would be worthwhile to note the occurrence of a multi-voltine relative of the Emma field cricket in the southern portion of Japan. This is the Taiwan field cricket, the species status of which has been discussed elsewhere (OHMACHI and MATSUURA, 1951a ; OHMACHI and MASAKI, 1964). It is found sporadically on the coast areas of the Kii peninsula, the Pacific side of Sikoku and most parts of Kyusyu. In these areas, it is usually bivoltine, its song being heard in early summer and autumn. Towards the southern tip of Kyusyu, this species becomes more abundant — a situation just reversed to that of the Emma field cricket. On the island of Tanegasima, it is probably the commonest kind of all crickets in contrast to the rare occurrence of the Emma field cricket. This dominant species presumably repeats several generations a year in this island, for its song is recorded almost continuously from March to December, and occasionally even at the end of January (correspondence from S. Yamasita of Tanegasima). The great abundance of this species and the contrasting rarity of the Emma field cricket clearly illustrate the inferior fitness of the latter to the very warm environment. The two species are morphologically as well as ecologically very similar except for the conspicuous difference in their life cycle and song. The difference in their diapause behaviour should therefore be the major adaptive difference responsible for their different success. It is possible that the presence of this competitor hinders the southern extension of the Emma field cricket before the latter reaches its climatic limit of tolerance, but the disadvantage of its long life cycle may be important in determining the outcome of such a competition.

It appears that a different factor or factors are operative at the northern limits of the Emma field cricket. As briefly stated before, the short growing season and the scarce sum of effective temperatures are evidently the limiting conditions, and the main problem is here the variability in the rate of maturation. So long as the obligatory

diapause concerns, there is no conceivable reason why the Emma field cricket fails to invade north beyond the 44°N latitude in Hokkaido. A recent experience in Hokkaido may well illustrate this point. In this northern island, abnormally cool weathers prevailed throughout the summer in 1964. At the end of August of this year, when many adults of the Emma field cricket were singing at Hirosaki, a collecting trip was made to Hokkaido for a week. During the trip no song of the Emma could be heard, though the Yezo field cricket, which extends north beyond the limits of the Emma in this island was singing at several sites. In an acquainted habitat at Sapporo, a number of Emma specimens were captured ; they were all immature except one female. This was indeed only one adult which could be collected throughout this trip. In normal years, the late summer is the best season for collecting adults at Sapporo. In view of the low temperatures prevailing in the autumn, it seemed that many failed to mature in this season. Although there is an adaptive variation in the developmental rate of the nymph, the variability is apparently set limits by some factors. The different physiological traits are thus responsible for the northern and the southern limits of the distribution.

Thus far the geographic variation in diapause and development of the Emma field cricket has been discussed in relation to the adaptation and distribution of the species. These two problems are different aspects of the same phenomenon which involves still another important facet, divergence of local populations. Before going farther into this last subject, it should be pointed out that the diapause behaviour of insects is characterized by the mode of incidence of diapause, the intensity or other traits relating to the completion of diapause, and the stage of development at which diapause supervenes.

As cited before, there are a number of instances of intra-specific variation in respect with the first two features of the diapause character. The local populations of a species may be variable in the intensity of diapause. If such a variation is not associated with a variation in the incidence of diapause, the adaptability of the species is confined within the framework of its constant voltinism as found in the Emma field cricket. Consequently, the life cycle of local populations is phenologically constant or changes only gradually so that the breeding activity occurs almost synchronously in contiguous populations. The free interchange of genes between populations is thus ensured so far as the life cycle concerns. The variation itself would not enhance further divergence of local populations.

The local populations may also be variable in the incidence, as well as in the intensity, of diapause. Some may enter diapause obligatorily, others facultatively, and still others may not enter diapause at all. In this case, a gradual polygenic shift might be involved as well, though the variation is phenotypically more or less discontinuous. Individuals with a genotypic capacity of facultative diapause may enter diapause or avert it in response to external conditions. The continuity or discontinuity of the genotypic variation would, therefore, depend on the phenotypic plasticity as well as on the genetic variability in the reaction threshold of the facultative diapause. On the one hand, intrinsic variations in the incidence of diapause may thus be concealed even within the same local population, and strains with particular diapause characters may be established by artificial selection (PREBBLE, 1941; HARVEY, 1957 ; COUSIN, 1961 ; SLIFER and KING, 1961). On the other hand, there are a few instances showing that populations with different voltinism are more or less isolated. In the plum curculio, *Conotrachelus nenuphar*, the cross between northern diapause females and southern non-diapause males shows a greatly reduced fertility, though the reversed cross is quite normal as compared with the intra-population matings (STEVENSON and SMITH, 1961).

In spite of this reduced fertility, the hybrid progeny are not sterile, thus the reproductive isolation is yet at its incipient stage. In *Teleogryllus commodus* of Australia, the genetic incompatibility seems to be complete between the southern diapause and northern non-diapause populations (BIGELOW and COCHAUX, 1962).

The stage of diapause in the life history is variable among different species, and even very closely related species often enter diapause at quite different stages of development. However, there are perhaps very few instances of intra-species variation of this sort. There must be reason for this constancy of the diapause stage within a species. One reason may be that a shift of diapause from one to another stage of development would require a drastic change in many physiological traits in order to adjust the life cycle to the seasonal changes. Comparison between the life cycles of the Emma and Taiwan field crickets will illustrate this point.

As already referred to before, the latter is multi-voltine, overwinters as a late instar nymph and no diapause occurs in the egg stage. Since the egg diapause of the Emma field cricket becomes gradually intensified towards the southwestern portion of this country where the two species are sympatric, the physiological difference in the egg stage between them does not disappear, but on the contrary becomes larger towards the zone of their contact. As discussed elsewhere, the life cycle of the Taiwan field cricket is adapted better to subtropical climates than temperate, whereas the reverse is the case in the Emma. The ranges of their climatic tolerance overlap to a certain extent as evidenced by their overlapping distribution. Nevertheless, the conspicuous difference in their way of seasonal adaptation in the sympatric areas shows their quite different genotypic responses to the same environment. There are apparently severe restrictions on their adaptational possibility. Such restrictions are without doubt imposed by the different frameworks of their major adaptations. There is an impassable adaptational barrier that separates the two species.

Their different life cycles are consolidated by different photoperiodic responses. The nymphs of the Emma field cricket grow more rapidly in a short (11-12 hrs) than in a long (15-16 hrs) photoperiod and the southern strains are more sensitive to such effects than are the northern strains (unpublished observations). The long summer days tend to retard, and the short autumn days to accelerate, the development whereby the majority of nymphs would mature at the proper time in autumn. The univoltine life cycle of the Emma field cricket is thus moulded not only by its obligatory diapause but also by this short-day type of the photoperiodic response. On the other hand, the Taiwan field cricket shows a usual long-day type of the response. The nymphs grow rapidly in a long photoperiod while they enter diapause in a short photoperiod. A similar photoperiodic response may occur even in a univoltine cricket which hibernates in the nymphal stage. Thus, the nymphs of *Nemobius yezoensis* are retarded to grow by a short day-length while they resume to develop when transferred to a long day-length (MASAKI and OYAMA, 1963).

The above story tells how firmly are various physiological features of the species interwoven into the life cycle to form a co-ordinated system of seasonal adjustment. It follows therefore that the species integrity would probably be disrupted sooner or later, if a variation occurs which involves a shift of the diapause stage. This supposition becomes more significant when one recognizes the dichotomous presence of the cold-hardy stages in the Orthoptera, that has been pointed out by ALEXANDER and BIGELOW (1960). Most members of this group of insects in eastern North America hibernate either in the egg or the nymphal stage. The same is true on the opposite side of the

field crickets. The two species are morphologically so similar that they had been treated as one species for a long time. Even such an experienced taxonomist as CHOPARD (1961) failed to recognize them on the morphological basis alone. As judged from their present geographic distribution and climatic adaptation in this country, the divergence of the two species from the non-diapause ancestor might have taken place allopatrically; or, it might have occurred in different geological epochs. The data available on the taxonomy, distribution, variation, ecology and physiology of this group of insects are all too scanty in the adjacent regions of this country in order to give a more or less precise reconstruction of this evolutionary event. However, the arguments put forth here seem to offer a reasonable working hypothesis for future analyses of the problem.

SUMMARY

Living specimens of the Emma field cricket (*Teleogryllus* sp.) were collected from different areas throughout almost the whole range in the Japanese archipelago. Their progeny were obtained in the laboratory and the durations of their incubation period were measured in various conditions of temperature. Several local stocks were maintained for a few successive generations and were subjected to similar experiments in each generation.

In all local strains examined, the eggs entered diapause irrespective of the temperature at which they were kept. A conspicuous geographic variation was, however, found in the intrinsic duration of the incubation period, and this appeared to be based on genetic factors. The incubation period, including the diapause stage, at constant temperatures (20°, 25° or 30°C) tended to be longer from north to south. The competence of the eggs to develop at a temperature (15°C) near the developmental threshold was higher in the northern than in the southern strains. The time required for breaking diapause by cold exposure (5-10°C) was shorter, and the hatchability after exposure to $0 \pm 1^\circ\text{C}$ was higher, in the northern than in the southern eggs. Finally, the incubation period at a warm temperature after hibernation tended to be longer in the southern than in the northern strains. Despite these variations, there was no remarkable difference in the temperature coefficient for completing the incubation period among the local strains. The geographic variation was therefore mainly found in the duration of the incubation period or the intensity of diapause.

The geographic pattern of the variation was analysed by means of multiple regression analysis. The results of such analyses showed that the variation was mostly due to the regression of the incubation period on the latitude and partly on the altitude of the original habitat of the strains. This geographic trend was accounted for by the regression on certain climatic conditions, for instance, the daily maximum temperature during the overwintering period. Based on these results, the mode of natural selection exerted upon the characteristics of the local eggs was suggested. Also, implications of the findings were discussed in relation to the geographic adaptation, distribution and divergence of the species.

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Pacific ocean. In Japan, out of 44 species (including undescribed ones) of the Gryllidae 32 overwinters as eggs, nine as half-grown or late instar nymphs and only the remaining three that are found in unusual habitats have no definite hibernating stages (OHMACHI and MATSUURA, 1951b). Based on this wide-spread tendency, ALEXANDER and BIGELOW presented an appealing picture of the evolution of a pair of closely related species, *Gryllus veletis*, which hibernates as a nymph, and *Gryllus pennsylvanicus*, which hibernates as an egg, from the common non-diapause ancestor. They laid emphasis on differential selection which would have splitted the ancestral population into two entities hibernating in the egg and nymphal stages, respectively. The different hibernating stages of the daughter populations would have caused the subsequent difference in the seasonal life cycle and the time of breeding activity and resulted in the temporal isolation between them.

The comparison between the seasonal adjustment of the Emma and the Taiwan field crickets suggests that such divergence of the hibernating stage should be consolidated by the subsequent adjustment of the entire life cycle, and that the divergent populations require quite different means of seasonal regulation. The rates of development at various stages, the norm of response to temperature, day-length or other external stimuli should all be modified in order to time the different hibernating stages to occur at the correct time before winter. Unless all these or some other timing devices are properly incorporated into the life cycle, the presence of cold-hardiness at a particular stage would be of little survival value. Fluctuating environments might cause the resistant stage to encounter a wrong season. Divergence in the hibernating stage should therefore be accompanied by divergence in many facets of the physiological constitutions. It seems difficult to assume that drastic re-organization of the physiological make-up of this kind could take place without a breakdown of the species continuity. Irrespective of whether allopatrically or sympatrically the process occurs, the divergence in developmental physiology deserves special investigation in understanding the speciation phenomenon in insects, as rightly emphasized by BIGELOW (1958, 1960a, b, 1962).

No indication has been found in the geographic variation of the Emma field cricket that such a divergence in the life cycle may occur simply as a result of adaptation to local diversities of climatic conditions. The same is probably true in the Taiwan field cricket. The life cycles of these field crickets are in a sense well specialized. They have little evolutionary potentiality of giving rise to another life cycle, because such a shift should require simultaneous occurrence of directional changes in the complicated genetic systems controlling various parts of the seasonal regulation mechanism. Such a possibility is axiomatically negligible. It should be a less specialized form which has a great evolutionary potential. Most species of crickets now inhabiting the temperate regions are presumably well equipped with elaborate mechanisms of seasonal regulation in their life cycles, and species without any efficient timing device can probably thrive only in equatorial climates. It seems therefore that the different life cycles of the Emma and Taiwan field crickets would have been diverged either directly or indirectly from a non-diapause tropical form. In fact, a member of *Teleogryllus* [probably *Teleogryllus mitratus* (BURMEISTER)] which resembles very closely both of the Japanese species is found in southeastern Asia. This tropical form has no capacity of diapause at all stages of its development, being entirely unresponsive to photoperiod (unpublished observations). Although the species status of this one should be investigated further, its very occurrence does not contradict with the above assumption.

There is little doubt about the common ancestry of the Emma and the Taiwan

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