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DIVERGENCE OF PHOTOPERIODIC RESPONSE AND HYBRID DEVELOPMENT IN *TELEOGRYLLUS*

(Orthoptera : Gryllidae)*

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In the Japanese Islands there occur three species of large field crickets belonging to *Teleogryllus*. As illustrated in figure 1, *T. yezoemma* (Ohmachi et Matsuura) occupies the northern half of the country, *T. emma* (Ohmachi et Matsuura) thrives in the widest central areas, and *T. taiwanemma* (Ohmachi et Matsuura) is restricted to the warm southern parts. The southern limit of the last species is at present unknown, which throws some doubt on its distinctness from the closely related form occurring in southeastern Asia. This tropical relative is *Teleogryllus mitratus* (Burmeister) which has been described originally from Java. Under this name, indeed, the Japanese forms had been confused until their distinctness at the specific level was recognized by Ohmachi and Matsuura (1951). Although the reproductive isolation among the three Japanese species has been demonstrated conclusively by Ohmachi and Masaki (1964), the above question has remained to be investigated. Also, in order to infer the origin of these species, it is important to know their relationships to the tropical form. The main objective of the present work is to elucidate such relationships by cross-breeding tests.

Whatever these relationships might be, this series of *Teleogryllus* forms may offer suitable material for understanding the problem of climatic adaptation in various regions. The solution of such a problem seems to be essential in constructing the evolutionary pictures of their speciation and the formation of their present patterns of distribution. Divergence in climatic adaptation frequently accompanies divergence in the life cycle, and this may play an important rôle in reproductive isolation (Alexander and Bigelow, 1960; Bigelow, 1960a, b, 1962; Ohmachi and Masaki, 1964; Masaki, 1965). The occurrence of very closely related species with different seasonal life histories seems to support this assumption (see also Bigelow and Cochaux, 1962; Hogan, 1965).

One of the most important aspects of climatic adaptation is the photoperiodic regulation of the developmental cycle, though at present the problem has scarcely been analysed in the Gryllidae. The three Japanese species as well as the tropical form were therefore reared and their development was observed under controlled

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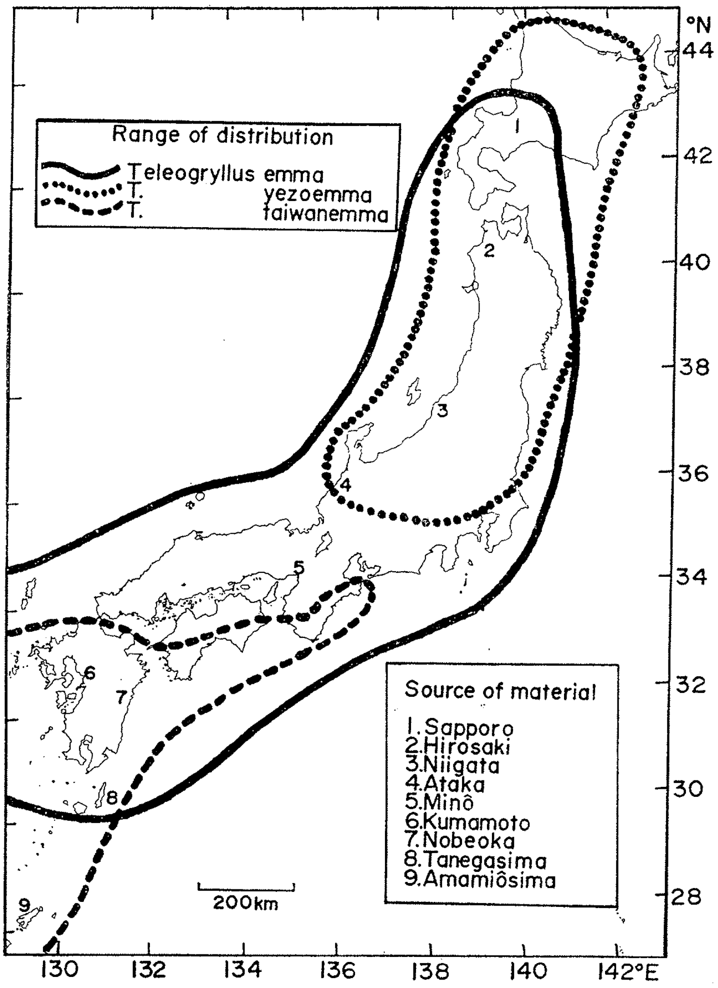

Fig. 1. Map showing the range of distribution of three species of *Teleogryllus* in the Japanese Islands and the original localities of stocks available for the present study.

conditions of photoperiod and temperature. If they exhibit diversified photoperiodic response, it is interesting to know the genetic relations of this physiological trait. Therefore, when the hybrids were obtained between the different forms, they were reared in different light regimes and their developmental characteristics were noted.

Materials and Method

Source of materials

The following stocks were available for the present study. *T. mitratus** from Djakarta (about 6°S, Java) and Pangkalan Brandan (about 3°N, Sumatra); *T. taiwanemma* from Amamiôsima (28°16′ N), Tanegasima (30°26′ N) or Nobeoka (32°34′ N, Kyusyu); *T. emma* from Kumamoto (32°46′ N), Minô (34°50′ N), Hirosaki (40°35′ N) or Sapporo (43°04′ N); *T. yezoemma* from Ataka (36°25′ N), Niigata (37°57′ N), or Asahikawa (43°45′ N). The map in figure 1 indicates the location of the collecting sites of the Japanese stocks.

The stocks of *T. mitratus* and *T. taiwanemma* were continuously reared at a high temperature, but those of the other two species were regularly subjected to cold during the egg stage in order to satisfy their diapause requirements. The progeny were thus maintained in the laboratory for several generations, during which cross-breeding tests are undertaken.

Crossing

In each crossing, several virgin females were kept together with a comparable number of males in a jar of five-litre capacity, in which a dish of moist sand was provided for oviposition. The jars were placed in room conditions during summer months and in incubators at 28°C during cold seasons. Under such conditions, many viable eggs were obtained in all the tested matings. The available combinations of species are given in table 1.

Table 1. *Combinations of crossing between* Teleogryllus *species referred to in the present study.*

♀ ♂	*mitratus*	*taiwanemma*	*emma*	*yezoemma*
mitratus	o	o	o	
taiwanemma	o	o	o	x
emma	o	o	o	x
yezoemma	o	x	x	o

o – crossing made in this work.
x – crossing described in Ohmachi and Masaki (1964).

Most eggs in every crossing hatched in the period comparable to the non-diapause egg stage, even when *T. emma* and *T. yezoemma*, both of which had obligatory egg-diapause, were mated with either species having no egg-diapause, *T. mitratus* or *T. taiwanemma*.

Conditions of rearing

The hybrid progeny as well as pure stocks were reared in boxes designed for photoperiodic treatment (Masaki, 1966b). Two different regimes were applied; daily photophases of 10 or 11 hours provided short-day conditions and those of

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* Official permit for the importation of living specimens and the subsequent laboratory rearing was given to S. M. by the Ministry of Agriculture and Forestry (Import permit No. 40–999).

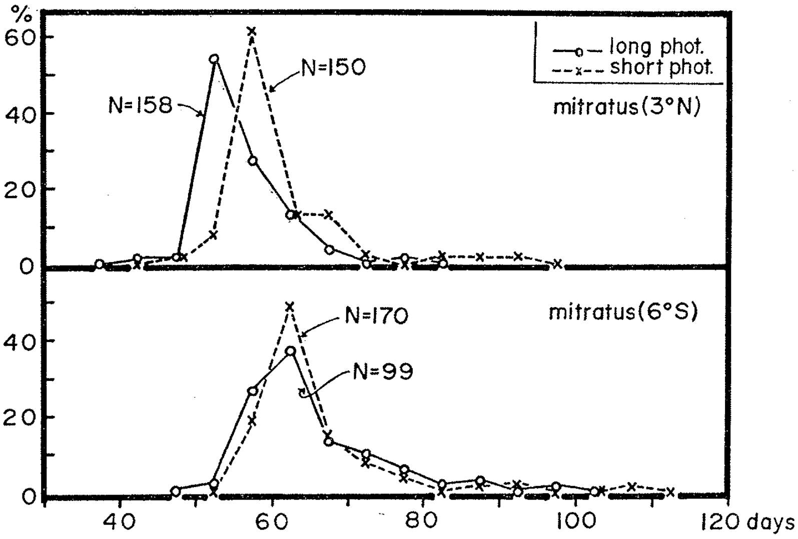

Fig. 2. Duration of nymphal development in two local strains (Sumatra, 3°N, and Java, 6°S) of *Teleogryllus mitratus*. Ordinate, number of adults; abscissa, time from hatching.

15 or 16 hours long-day conditions. Temperature in the boxes averaged about 26° to 28°C. In a few cases, hybrid crickets were reared in an ordinary incubator in which no light source was available. For further details of the rearing method, see Masaki (1966b).

Under these conditions of rearing, the time from hatching to emergence as an adult was recorded for each individual, and the frequency distributions of the developmental period were obtained. These data will be presented below mainly in diagramatic forms, since the characteristics of the species or peculiarities of hybrids in the nymphal development and photoperiodic response may be visualized most easily by such presentations. This is particularly so for hybrids developing in a highly variable fashion.

Results

Teleogryllus mitratus

The durations of nymphal development of this species are given in figure 2. In this figure, two strains originating from Sumatra and Java are represented. The Java strain developed at almost identical rates at the two photoperiods, and the mean developmental period was about 65 days. Under the same conditions, the strain from Sumatra completed development a few days earlier than this. The mean nymphal stages were 57 days in the 16-hour photoperiod and 61 days in the 11-hour photoperiod. It was not certain if this slight difference between the two means was really caused by the varied photoperiodic conditions.

Thus, these tropical populations are characterized by a virtual absence of photoperiodic response, as might be expected from the equatorial climate of their habitats. The monthly mean of temperature fluctuates between 26.2° and 27.4°C

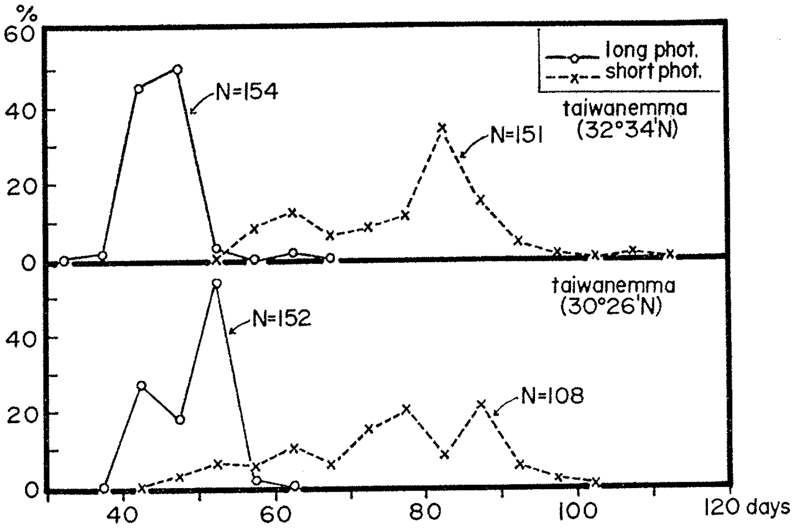

Fig. 3. Duration of nymphal development in two local strains (Nobeoka, 32°34′N, and Tanegasima, 30°26′N) of *Teleogryllus taiwanemma.*

at Djakarta and between 25.6° and 27.2°C at Medan near Pangkalan Brandan, the annual range being narrower than 2°C (the 1965 edition of Rikanempyô).

Teleogryllus taiwanemma

Figure 3 illustrates the photoperiodic response of this species that had been derived from the southern islands of Japan, Tanegasima and Kyusyu. In both strains, photoperiod exerted conspicuous effects on the development of nymphs. In the long-day regime, the nymphs of the Kyusyu and Tanegasima strains completed development, on the average, in 46 and 49 days, respectively. In the short-day regime, they required one further month and emerged as adults after mean periods of 78 and 75 days from hatching, respectively. In a later experiment, a stock collected from Amamiôsima was observed responding to photoperiod in a similar way.

Near the northern border of distribution of this species in the Japanese Islands, the annual temperature averages about 15°C and the two extremes of monthly mean are 5° and 27°C. South in the Ryukyus the climate is much milder with an annual mean higher than 20°C. Even there, however, the winter conditions are not always favourable for rapid development of this form, and the monthly mean is as low as 14.3°C in January at Naze. The seasonal variation of day-length attains to three hours or more in this latitude. It is not surprising, therefore, that these subtropical inhabitants are highly sensitive to photoperiod, and that their response belongs to the long-day type. Probably, this response controls the occurrence of facultative diapause in the nymphal stage, through which their multivoltine cycle is adapted to the seasonal changes of environmental conditions.

Teleogryllus emma

This species is apparently adapted to temperate climates (Masaki, 1965). Within its range, the annual mean temperature varies from 7° to 19°C. It has a univoltine

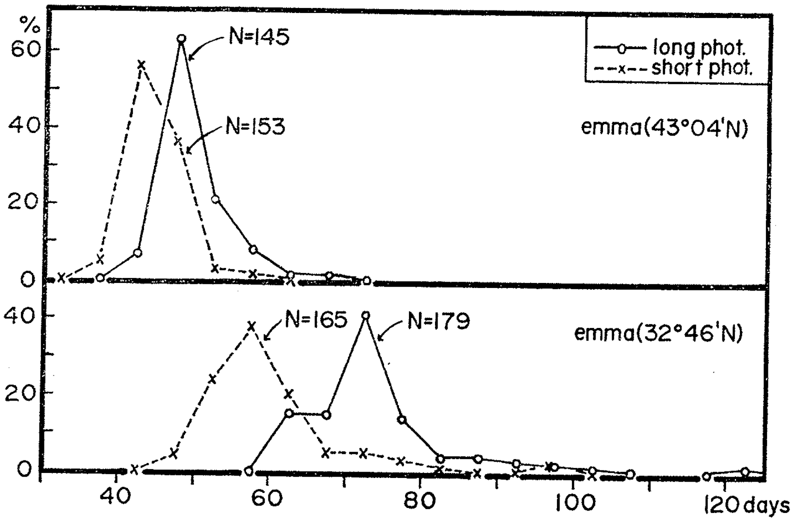

Fig. 4. Duration of nymphal development in two local strains (Sapporo, 43°04′N, and Kumamoto, 32°46′N) of *Teleogryllus emma.*

life cycle with an obligatory diapause in the egg stage. Despite this stabilized type of life cycle, the species responds to photoperiod by varying the timing of nymphal development (Masaki, 1966a). This is indicated in figure 4. In both the northern and the southern populations, the response may be termed as the short-day type, because the adults appeared in a shorter time in the short- than in the long-day conditions. This tendency was more pronounced in the south, and there was a northward decrease in the amount of photoperiodic response. The detail of this variation has been described in a previous paper (Masaki, 1966a).

The short-day type of response in this species is apparently responsible for the seasonal adjustment of its univoltine life cycle, and may be regarded as an adaptation to such regions where hot summers alternate with more or less severe winters.

Teleogryllus yezoemma

The life cycle of this species closely resembles that of *T. emma.* It is also regulated by a photoperiodic response of the short-day type. Figure 5 illustrates this. In the southern strain the nymphal development was prolonged to a considerable extent by the long photoperiod, but in the northern strain such an effect was less clear. The details of this photoperiodic response and its geographic variability have been the subject of a previous paper (Masaki, 1966b).

This species extends to the coldest parts of this country where the annual mean temperature slightly exceeds 5°C, and the southern limit coincides with the 14°C isotherm of annual mean. The basic type of adaptation of this species is doubtless similar to that of *T. emma* and the difference in their ranges of distribution might be related to some factors other than the photoperiodic response.

In the foregoing accounts, it has been demonstrated that the species inhabiting different climatic regions have definitely different means of seasonal regulation.

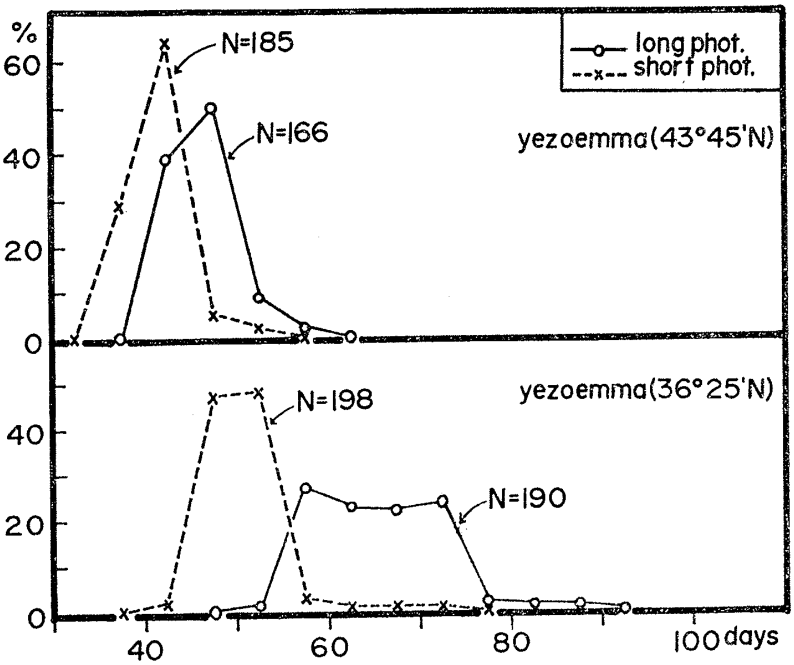

Fig. 5. Duration of nymphal development in two local strains (Asahikawa, 43°45′N, and Ataka, 36°25′N) of *Teleogryllus yezoemmma.*

The tropical form lacks in the ability of photoperiodic response, the subtropical form is able to repeat generations or to enter facultative diapause in response to the seasonal changes of day-length, and the two northern species stabilize their univoltine life cycle through their characteristic responses of the short-day type.

T. taiwanemma ♀ × *T. mitratus* ♂

In the progeny derived from crossing between *taiwanemma* females from Tanegasima and *mitratus* males from Sumatra, no male adults occurred for a period of 150 days or more. Many male nymphs persisted at the end of this period, but none reached the penultimate instar. The females developed normally (figure 6, upper); the mean duration of nymphal stage in the long photoperiod was 52 days, being intermediate between the growing periods in the same sex of the parental stocks, 47 days in *taiwanemma* and 56 days in *mitratus*, under the same conditions. The growing stage of the hybrids was prolonged to 63 days by the short photoperiod. This was longer than that of the *mitratus* females, but shorter than that of the *taiwanemma* females. Therefore, long-day type of response of the female parent was manifested, though in a decreased degree, in these hybrid progeny.

The results were essentially similar when *mitratus* males from Java were crossed to *taiwanemma* females from Tanegasima (figure 7, upper). The male progeny were again retarded to develop, and only three male adults were obtained after 326, 342 and 391 days of rearing. The rest died before the penultimate instar. As in the previous cross, the female offspring developed normally. In the long pho-

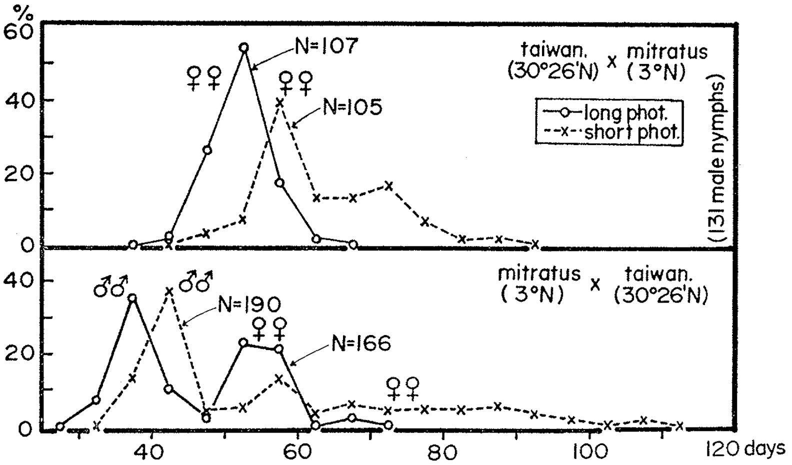

Fig. 6. Duration of nymphal development in reciprocal hybrids between the Tanegasima strain of *T. taiwanemma* and the Sumatra strain of *T. mitratus.*

toperiod, the nymphal stage averaged about 55 days as compared with 49 days in the pure Tanegasima stock and 59 days in the Java stock. Under the short-day conditions, the growth periods of the parental stocks were identical, averaging about 65 days, and the hybrids also matured in 66 days.

Other groups of hybrids from the same interspecific mating were reared in darkness and similar results were obtained. It was found, however, that the origin of the parent females seemed to affect to a certain extent the development of male hybrids (figure 8). When *taiwanemma* females from Kyusyu were crossed to *mitratus* males, a few hybrid males reached the adult stage. These males developed at an abnormally high rate. The majority of males developed very tardily and remained at very early stages when all females had completed growth. These retarded nymphs usually died off before their wing pads taking the dorsal position.

Rearing in darkness was not the cause of the occurrence of a few fast-growing males. Under the same conditions, the hybrids between the Tanegasima stock of *taiwanemma* and the Sumatra stock of *mitratus* did not yield such males, though a few male adults appeared between 199 and 282 days after hatching. The rest died before reaching maturity.

T. mitratus ♀ × *T. taiwanemma* ♂

This crossing, the reverse of the above, produced progeny of quite different characteristics (figure 6, lower). When Sumatra females of *mitratus* were mated with Tanegasima males of *taiwanemma*, adult males and females occurred in about equal numbers in the progeny. Moreover, the males developed very fast; the male nymphal stage averaged about 39 and 41 days in the long and short photoperiods, respectively. The comparable means were 51 and 79 days for the Tanegasima males of *taiwanemma*, and 57 and 62 days for the Sumatra males of *mitratus*. Therefore, the hybrid males developed faster than did either parental species. This might be another expression of genetic disturbance caused by hybridization. At the same time, the physiological system of photoperiodic response

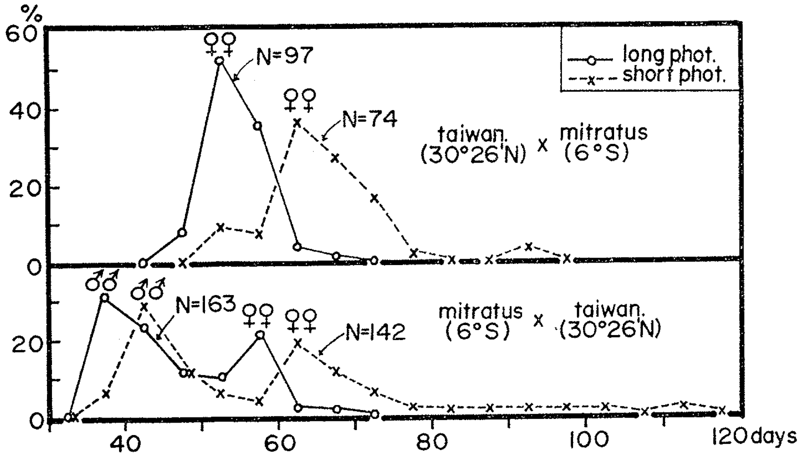

Fig. 7. Duration of nymphal development in reciprocal hybrids benween the Tanegasima strain *T. taiwanemma* and the Java strain of *T. mitratus*.

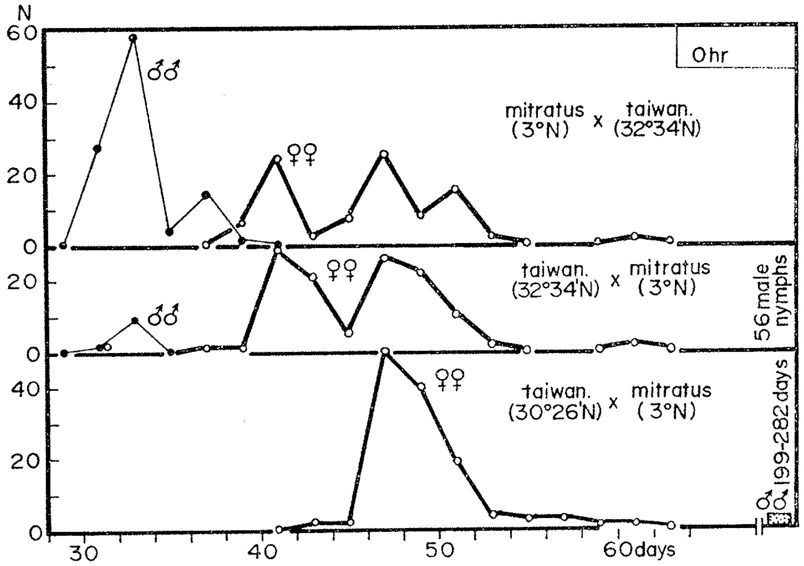

Fig. 8. Duration of nymphal development in hybrids beween *T. taiwanemma* and *T. mitratus*; the local strains were derived from Sumatra (3°N), Tanegasima (30°26′N) and Nobeoka (32°34′N).

might be upset, because there was only two days' difference in developmental time between the long-day and short-day groups.

As in the reversed cross, the hybrid females developed normally, and their nymphal stage was almost identical to the male parent, the Tanegasima stock of *taiwanemma*. Their photoperiodic response was also similar to the Tanegasima stock, and a delay of about 23 days in maturation time was induced by the

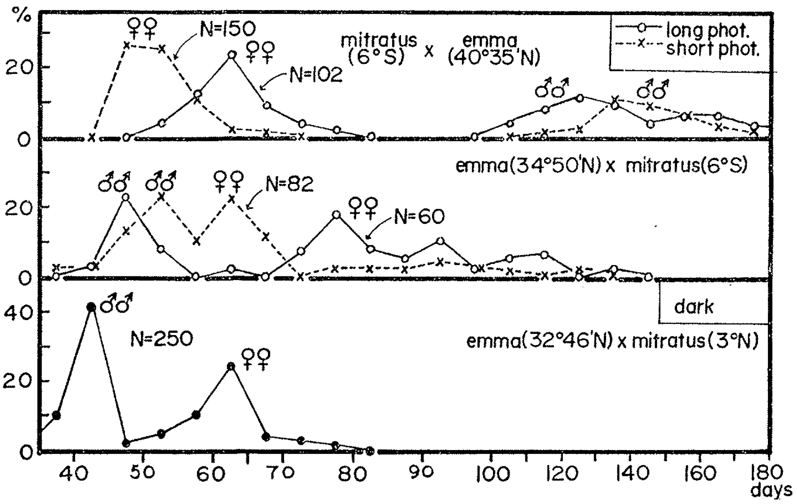

Fig. 9. Duration of nymphal development in reciprocal hybrids between *T. mitratus* and *T. emma*. The local strains were derived from Java (6°S), Hirosaki (40°35′N), Minô (34°50′N) and Kumamoto (32°46′N).

change of photoperiod from 16 to 11 hours.

These results were confirmed by crossings between *mitratus* females from Java and *taiwanemma* males from Tanegasima (figure 7, lower) or between *mitratus* females from Sumatra and *taiwanemma* males from Kyusyu (figure 8, upper). In the hybrid progeny of these crosses, the curve of adult emergence showed two distinct peaks without exception; the first one represented the emergence of male adults and the second that of female adults.

T. mitratus ♀ × *T. emma* ♂

In this crossing, the Java strain of *mitratus* was used as female parent and the Hirosaki strain of *emma* as male parent. The female hybrids developed normally, and on the average reached maturity 63 and 52 days after hatching in the long and the short photoperiods, respectively. The short-day type of photoperiodic response was thus inherited from the male parent (figure 9, top).

The male hybrids developed at a slow rate, but their development was not entirely suppressed and they emerged as adults between 100 and 200 days after hatching. As a result the periods of adult emergence of the two sexes were completely separated (figure 9, top). The ultimate adult sex-ratio was nevertheless close to unity, which means that the males did not suffer high mortality despite the retarded development. There were thus 230 males and 238 females in the two photoperiodic groups. The photoperiodic response of male hybrids was obscure. It was not certain that this was due to the greatly extended and variable duration of development or to upsetting of the physiological system involved.

T. emma ♀ × *T. mitratus* ♂

Females from the Minô stock of *emma* and males from the Java stock of *mitratus* were available for this cross. The progeny were quite different from the reversed cross, and showed a close resemblance in their developmental characteristics to the hybrids between *mitratus* females and *taiwanemma* males. Namely, males emerged first and females followed this (figure 9, middle). The mean durations of male nymphal stage were 50 and 52 days in the long and short photoperiods, respectively. The photoperiodic response was thus not manifested in the male hybrids, as in the case of *mitratus* ♀ × *taiwanemma* ♂. Moreover, the developmental time was considerably shorter than that of either parental stocks. The male *emma* of the parental stock required 73 and 57 days in the long and short photoperiods, respectively; the male *mitratus* of the parental stock completed development in 63 days at either photoperiod.

Most female hybrids developed at a normal rate, but a few required a prolonged period to mature. This was indicated by the large value of standard deviation, about three to four times as large as those for males. The short-day type of photoperiodic response was clearly seen in the emerging dates of female hybrids, which were on the average 90 and 71 days after hatching at the long and short photoperiods, respectively.

Progeny obtained by crossing between *emma* females from Kyusyu and *mitratus* males from Sumatra were reared in darkness at 29°C, and the results were similar to those stated above (figure 9, bottom). The male hybrids completed development, on the average, in 42 days while the female hybrids in 62 days, there being 20 days' time lapse between the peaks of emergence of the two sexes.

T. emma ♀ × *T. taiwanemma* ♂

The nymphal development of this hybrid in darkness was described previously (Ohmachi and Masaki, 1964), but the photoperiodic response has been unknown. Figure 10 (upper) illustrates the emergence curves of the hybrids in the long and short photopeirodic conditions. The parental stocks of *emma* and *taiwanemma* in this cross were derived from Minô and Tanegasima, respectively.

As shown in the figure, a small number of hybrid males appeared first in mean periods of 47 days in the long photoperiod and 49 days in the short photoperiod. This was followed by the appearance of female adults. Any photoperiodic effect could not be observed on the emergence of males, but a clear-cut response was found in the female. The mean duration of female nymphal stage was 70 days in the long photoperiod, while it was as long as 94 days in the short photoperiod. Therefore, the female hybrids were more strongly affected by the long-day type response of their male parent than by the short-day type response of the female parent. However, the influence of female parent was not entirely masked. This was indicated by the fact that the nymphal stage in the long photoperiod was almost comparable to that of the female parent, about 73 days, and much longer than that of the male parent, 45 days.

A further point to be stated is that, in the short photoperiod, the developmental period of the hybrid females was longer than that of either parental species. As stated above, the mean was 94 days in the hybrids, while it was 79 days in *taiwanemma* and 56 days in *emma*. However, the difference in developmental time between the two photoperiodic groups was a little smaller in the hybrids than in the pure stocks of *taiwanemma*.

Although only a small number of hybrid adults were obtained, the result was

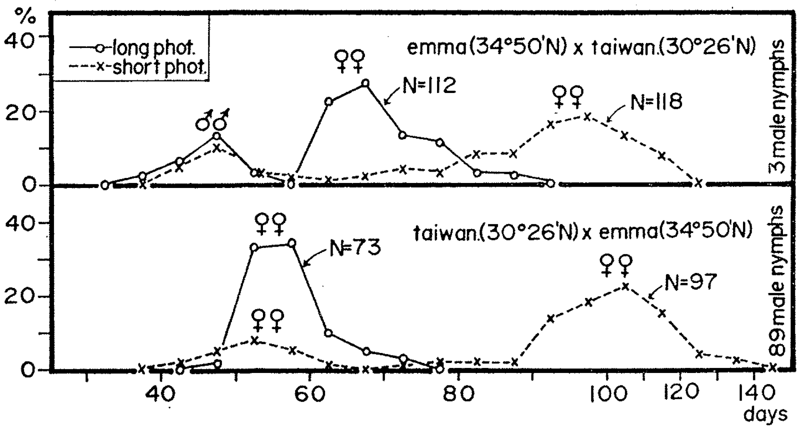

Fig. 10. Duration of nymphal development in reciprocal hybrids between the Tanegasima strain of *T. taiwanemma* and the Minô strain of *T. emma*.

similar when the *emma* parent was derived from Hirosaki. Only 7 males and 26 females emerged as adults in the long photoperiod, and their mean durations of nymphal stage were 44 and 61 days, respectively. In the short photoperiod, no male adults occurred; the mean periods of development of 11 females was 88 days, about four weeks longer than in the long photoperiod.

T. taiwanemma ♀ × *T. emma* ♂

This crossing was characterized by absence of male adults among the progeny, as described previously (Ohmachi and Masaki, 1964). The male nymphs developed tardily, many survived for several months, but none reached the penultimate instar. When *emma* males from Minô were crossed to *taiwanemma* females from Tanegasima, the mean durations of development of the resulting hybrids were 58 and 96 days in the long and the short photoperiods, respectively (figure 10, lower). When *emma* males from Hirosaki fertilized females of the same stock of *taiwanemma*, the duration of nymphal development in the progeny was a little shorter than this, 52 days in the long photoperiod and 90 days in the short photoperiod. Again, no male adults were obtained.

There was thus a conspicuous response of the long-day type in the progeny of these crossings. Under the short-day conditions, the developmental delay in the majority of hybrid crickets was even greater than that usually observed in the pure stocks of *taiwanemma*.

Comparison of these data with those of the reversed cross reveals a complicated genetic feature of the photoperiodic response. There was no conspicuous difference between the reciprocal matings in the developmental rate of offspring females under the short-day conditions. The mean durations of development were 96 and 94 days in the reciprocal hybrids between the Minô *emma* and the Tanegasima *taiwanemma*, and 90 and 88 days in those between the Hirosaki *emma* and the Tanegasima *taiwanemma*. On the other hand, under the long-day conditions, the reciprocal matings yielded significantly different results. The hybrid means were 58 and 70 days when the males and females of Minô *emma* were mated

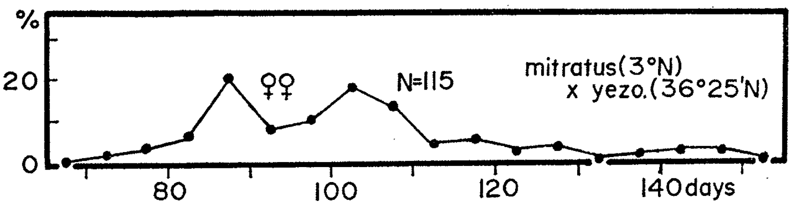

Fig. 11. Duration of nymphal development in hybrids between Sumatra females of *T. mitratus* and Ataka males of *T. yezoemma.*

with the opposite sexes of *taiwanemma,* respectively, and 52 and 61 days when the males and females of Hirosaki *emma* were mated with the opposite sexes of *taiwanemma,* respectively. This may suggest that the short-day type of response, i. e., prolongation of development under long days, of *emma* was more strongly manifested when this species participated in the mating as mother than as father. At the same time, the progeny were affected by the long-day type response of *taiwanemma* and grew at a slow rate in the short photoperiod.

In discussing the difference in photoperiodic response as an isolating mechanism between *taiwanemma* and *emma,* Ohmachi and Masaki (1964) simply supposed that the response might be controlled by a polygenic system, and therefore that the hybrids between these species would have a response of decreased efficiency because of their intermediate character. According to the present data, this supposition is not correct and the hybrids are more strongly affected by the long-day type response of the *taiwanemma* parent. Other factors such as the difference in song or hybrid sterility may be more important in preventing the gene interflow between sympatric populations of the two species.

T. mitratus ♀ × *T. yezoemma* ♂

Both sexes suffered retarded development in the progeny of this crossing, though in different degrees. No male adults were obtained. The female hybrids also developed slowly with highly variable rates, but they attained to the adult stage. When males of the Ataka stock of *yezoemma* were mated with females of the Sumatra stock of *mitratus,* the offspring nymphal stage ranged from 75 to 181 days with a mean as large as 102 days (figure 11). The result was similar when the cross was made between Java females of *mitratus* and Niigata males of *yezoemma.*

These results were obtained at a high temperature (29°C) in darkness. The photoperiodic response was not examined because of the difficulty to assess such effects on this long and variable period of development.

Fertility of hybrids

Most hybrids referred to above were not fertile except for those produced by the reciprocal matings between *mitratus* and *taiwanemma.* When infertile hybrid females had been reared for several weeks, it was usual that their abdomen became much distended as if their ovaries were full of mature eggs. The dissection revealed, however, that they had in most cases no or little mature eggs, and that the distended abdomen was due to the enormously hypertrophied fat body. The situation resembled that of allatectomized females of certain insects (Wigglesworth, 1954).

Table 2. *Cross-breeding for testing the fertility of hybrids between* T. mitratus *and* T. taiwanemma.

♀♀	n	♂♂	n	Duration of oviposition	Number of nymphs
t	20	t	10	13 days	7, 856
m	20	m	10	13	1, 339
t	30	m	15	21	5, 731
m	20	t	13	12	1, 708
t	3	m × t	5	20	1, 161
m	16	m × t	15	20	1, 456
t × m	10	m	9	20	3, 982
t × m	19	m × t	18	20	6, 769
m × t	38	m × t	33	20	10, 387
m × t F_2	26	m × t F_2	18	16	9, 204

t: *taiwanemma.* m: *mitratus.* n: number of adults.

Fertility of hybrids between *mitratus* and *taiwanemma* was tested by various combinations of mating as arranged in table 2. From all the crosses noted in the table, viable offspring were produced. Therefore, both sexes of the hybrids between *mitratus* females and *taiwanemma* males were fertile, and the females descended from the reversed mating were also fecund.

Development of F_2 *and* F_3 *hybrids*

The development of the progeny descended from the hybrids between *mitratus* and *taiwanemma* was not quite normal (figure 12). In the F_2 progeny, the females completed development on the average in 54 and 59 days at the long and short photoperiods, respectively, and the males in 83 days at both photoperiods. Therefore, there was about 20–30 days' difference between the mean dates of emergence of the two sexes. The emerging periods of males and females broadly overlapped, and the difference between the means was mainly due to the greater variance in the male. In fact male adults continued to appear from 40 to 400 days after hatching. In spite of this, the sex ratio was not greatly affected. There were 258 male adults and 320 female adults in the two photoperiodic groups. A slight photoperiodic response might be observed in the female, as a few females showed delayed development at the short photoperiod but none did so at the long.

The F_2 progeny of *mitratus* × *taiwanemma* were fertile and produced F_3 progeny (table 2, bottom). The latter were reared in darkness at 28°C and adults of 204 males and 180 females were obtained. There was little difference in the mean periods of development between the sexes, 57 days for the male and 56 days for the female. However, the variance was again very large in the male, of which the period of adult emergence ranged from 38 to 411 days (figure 13). The females also showed a wide range of variation as compared with the pure stock and the same sex of F_1 hybrids, requiring from 38 to 235 days to mature.

A small group of backcross progeny, *taiwanemma* ♀ × (*mitratus* ♀ × *taiwanemma* ♂) ♂, was reared in darkness at 28°C (figure 14). The females developed rapidly and the mean period of nymphal stage for 31 specimens was 42 days. The nymphal development in the male was delayed and highly variable. Male

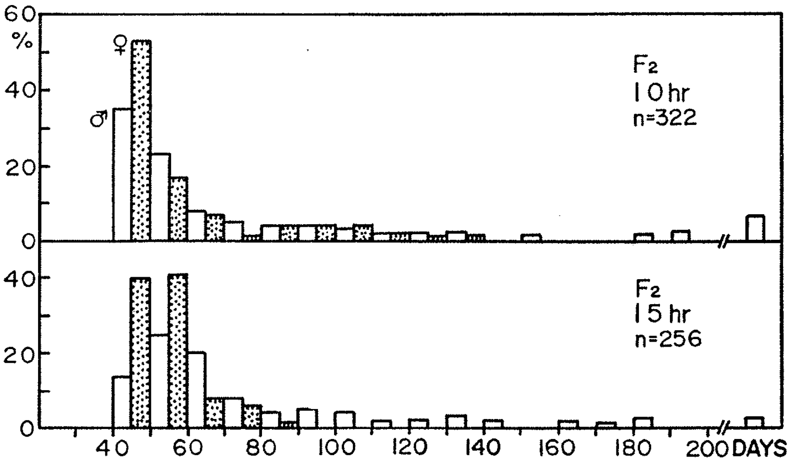

Fig. 12. Duration of nymphal development in F_2 hybrids between *T. mitratus* and *T. taiwanemma.*

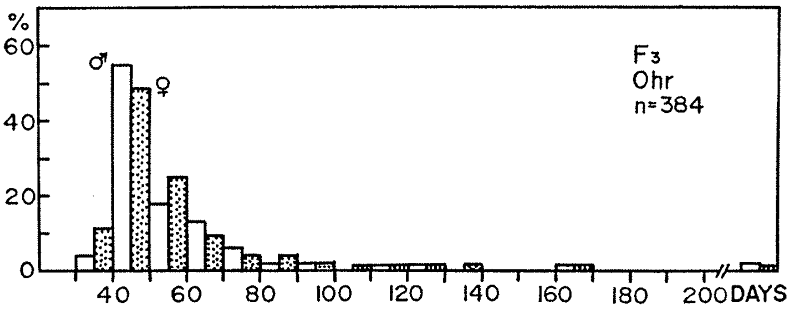

Fig. 13. Duration of nymphal development in F_3 hybrids between *T. mitratus* and *T. taiwanemma.*

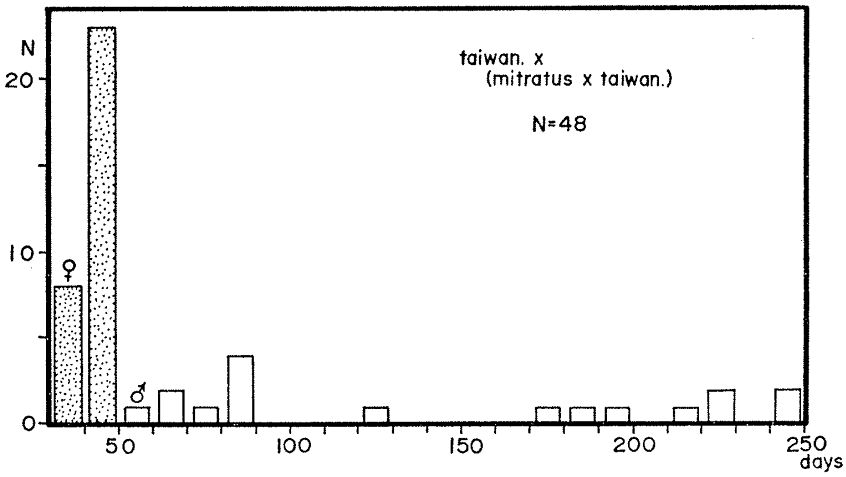

Fig. 14. Duration of nymphal development in the backcross progeny, *T. taiwanemma* ♀× (*T. mitratus* ♀ × *T. taiwanemma* ♂) ♂.

adults began to appear from 58 days after hatching and thereafter emerged sporadically until 256 days. The mean growing period was 143 days, and there were only 17 males in all. Some kind of segregation seemed to occur as to the developmental retardation in the male, since there was, as described above, no such delay in development of males in the F_1 generation of *mitratus* ♀ × *taiwanemma* ♂.

Crossing between local strains

All hybrids described above were not normal in that the males in most cases and both sexes in a few cases showed abnormal rates of nymphal developmnet. On the other hand, such disturbances in nymphal development were not observed when different local strains of the same form were crossed with one another. The reciprocal crosses between the Java and Sumatra strains of *mitratus* yielded offspring the both sexes of which developed normally. Likewise, the Nobeoka and Amamiôsima strains of *taiwanemma* produced hybrids which were not affected by any kind of disturbed development. As reported in a previous paper (Masaki, 1963), the Sapporo (43°04′ N) and Kôti (33°33′ N) strains of *emma* distinctly differ from one another in growth rate and body size, but both sexes of the hybrids between them were normal in that they were approximately intermediate in growth rate between the parents. Crossing between different local strains of *yezoemma* has not been undertaken as yet.

Discussion

The close kinship between the tropical and Japanese forms of *Teleogryllus* has been substantiated by their ineterfertility. It is highly probable that they have been derived from a common ancestor in relatively recent geological times. Their close relationships are also suggested by their morphological similarity. It should be emphasized, however, that they are well differentiated in developmental physiology. Thus, they represent distinct types of seasonal adaptation and have different ranges of distribution. The tropical field cricket, *T. mitratus,* is a homodynamic species, neither diapause nor photoperiodic response of any kind being recognized in its development; the subtropical form, *T. taiwanemma,* has a multivoltine life cycle and enters a nymphal diapause which is controlled by a photoperiodic response of the long-day type; and the two northern species, *T. emma* and *T. yezoemma,* obligatorily enter diapause in the egg stage and consequently are univoltine. Their photoperiodic responses are characterized by the short-day type. Despite the similarity of the last two species, the genetic bases of their seasonal regulation may not be identical. Intercrossing between them gave an unexpected result; diapause was remarkably abbreviated in the hybrid eggs, some of which completely failed to enter diapause (Ohmachi and Masaki, 1964). If this abbreviation of diapause is due to the heterozygous conditions of the diapause genes in the hybrids, it seems possible to assume that the egg-diapause has been evolved in the two species independently of one another.

Thus, all the four forms of *Teleogryllus* seem to have their own characteristic genetic bases of climatic adaptation. Undoubtedly, this divergence in developmental physiology has been the main factor in establishing their different geographic ranges and might have been an important momentum for their further divergence. The different types of climatic adaptation would have decreased gene interflow between the diverging forms and enhanced isolation at some stage of their evolution.

Although at the present state of our knowledge it is difficult to reconstruct a concrete picture of the divergence of the diapause behaviour and photoperiodic response of these crickets, one possibility may be suggested. That is, the seasonal life cycles of the three Japanese forms might have been derived directly and independently of one another from the non-diapause tropical species, *T. mitratus* itself or its predecessor. This seems to be the only possible hypothesis that can be derived from the known facts.

First, there is no evidence showing the existence of a direct link between the two northern species, *T. emma* and *T. yezoemma*, since, as noted above, their similar life cycles are presumably controlled by different genetic bases. It is then possible to assume that they have acquired their obligatory egg-diapause on different occasions and subsequently invaded or extended over the temperate region from the different places of origin in different geologic times.

Second, there is also no evidence to suppose that the univoltine life cycle with an obligatory egg-diapause of these two species could have been evolved from the multivoltine life cycle of *T. taiwanemma.* If the northern segment of the latter should respond to selection pressure exerted by a cool climate, it would be easier to evolve a univoltine life-cycle with an obligatory nymphal diapause than to shift diapause from the nymphal to the egg stage. The occurrence of univoltine crickets overwintering in the nymphal stage suggests the possibility of this evolutionary response. *Nemobius yezoensis* in the Japanese Islands (Masaki and Oyama, 1963), *Gryllus campestris* in Europe (Fuzeau-Braesch, 1966), and *Gryllus veletis* in North America (Alexander and Bigelow, 1960) are such examples. Although this has not been established for unknown reason and the species is restricted to the warmer parts of the country, such a change may be induced by a gradual shift of the critical photoperiod toward a longer range. There are in fact many examples of the latitudinal cline of the critical photoperiod in various insects (Danilevskii, 1961).

On the other hand, as discussed previously, there is an impassable adaptational gap between the nymphal and egg overwintering (Masaki, 1965). Unless a shift of diapause from the nymphal to the egg stage is accompanied by a simultaneous variation of photoperiodic response from the long-day to the short-day type, the seasonal regulation mechanism would lose its efficiency. It seems unlikely that such a shift might be completed at a single step, because the results of crossing experiments show that the egg-diapause and the photoperiodism in nymphal development are controlled by different genes. Moreover, in view of the dichotomous occurrence of the cold-hardy stages in the Orthoptera (Alexander and Bigelow, 1960), it is not feasible to imagine a continuous or gradual change from one to the other type of overwintering.

There may be no such adaptational difficulties when a diapause or photoperiodic response of any kind is evolved first in a non-diapause population. The occurrence of diapause or cold-hardiness at either the nymphal or egg stage would be advantageous if the population is suffering changes from warm to cooler climates, because it would simply increase the tolerance to the adverse environment. Likewise, the acquisition of photoperiodic response would be advantageous, since it would synchronize development of individuals. If any kind of diapause is combined with an appropriate type of photoperiodic response, the seasonal regulation mechanism becomes more efficient. On the other hand, if there occurs a wrong combination, it would be eliminated at once. For example, a short-day type of photoperiodic response combined with a facultative nymphal diapause is obviously fatal, because it induces diapause in summer but not before winter.

In order to infer further details of the variation involved in this divergence, the genetic analyses of diapause and photoperiodism are necessary. Unfortunately, the present data provide little information about this.

The egg-diapause of *emma* and *yezoemma* failed to manifest itself when these species were crossed to the species having no egg-diapause. This was observed previously by reciprocal crosses between *emma* and *taiwanemma* or between *yezoemma* and *taiwanemma* (Ohmachi and Masaki, 1964), and also confirmed in the present work by reciprocal crosses between *emma* and *mitratus* and the cross between females of *mitratus* and males of *yezoemma*.

The long-day type of response of *taiwanemma* was manifested in the reciprocal hybrids with *mitratus*. The reciprocal hybrids between *emma* and *mitratus* were similar to *emma* in that they showed the short-day type of nymphal development. In both cases, however, the amount of response by the hybrids were variable, and there seems to be no simple dominant-recessive relationship as to the manifestation of photoperiodic response. When reared in a short photoperiod, the nymphal diapause of *taiwanemma* persisted conspicuously in the progeny of matings with *emma*. In this case the growing periods of the reciprocal hybrids were different in a long photoperiod, though identical in a short, and so the maternal effect and the environmental modification for the manifestation of the genetic traits may be presumed.

Most hybrids were not fertile, so that it was impossible to analyse details of the genetic relationships of the various photoperiodic responses and diapause behaviours. Moreover, hybrid males were without exception not normal in their development. Similar phenomena of disturbed development partly occurred also in the F_2 and F_3 hybrids between *mitratus* and *taiwanemma*. If such disturbances were not to occur, they should provide useful information on the genetics of photoperiodism.

The developmental disturbance was mainly manifested in either the accelerated or retarded growth in the male. In extreme cases the males failed to mature and died as nymphs. Except for hybrids between *mitratus* and *yezoemma*, female hybrids were more or less normal in as much as they were intermediate between the parents or almost identical to either parent in their developmental velocity. Since the male is the heterogametic sex in the Gryllidae (Ohmachi, 1935), the Holdane's rule seems to hold in these cases. The situation is very similar to that found in the hybrids between certain species of field crickets of North America (Bigelow, 1960a). This may be due to deleterious effects of interspecific combinations of autosomal and sex-chromosomal genes. Bigelow inferred further that the genes responsible for the retarded development and located on the sex-chromosome were recessive, for hybrid females, having a heterozygous pair of sex-chromosomes, did not show any retardation. Segregation in such combinations of autosomes and sex-chromosome might be expected to occur in the F_2 or back-cross generations, and the highly variable durations of development of males in these generations of *mitratus* ♀ × *taiwanemma* ♂ might be accounted for by this assumption. The occurrence of retarded females in the F_3 generation of the hybrid line, which was not observed in the F_1 and F_2, provides further evidence in favour of this opinion, because in this generation some females should have a homozygous pair of X-chromosomes derived from *taiwanemma* together with some autosomes of *mitratus*. It should be remembered that the male hybrids, *taiwanemma* ♀ × *mitratus* ♂, were retarded to grow and that they had the X-chromosome of *taiwanemma* together with the heterozygous pairs of autosomes.

Table 3. *Various degrees of abnormality in the sex-ratio and nymphal development of hybrids between* Teleogryllus *species.*

Sex ratio	Development		Crossing
	♂	♀	
+	(+)	+	*mitratus* × *taiwanemma** *emma* × *mitratus*
+	±	+	*mitratus* × *emma*
±	(+), −	+	*emma* × *taiwanemma* (*taiwanemma* × *mitratus*)*
−	−	+	*taiwanemma* × *mitratus** *taiwanemma* × *emma* *emma* × *yezoemma* (Ohmachi and Masaki, 1964) *yezoemma* × *emma* (Ohmachi and Masaki, 1964)
−	−	±	*mitratus* × *yezoemma* *taiwanemma* × *yezoemma* (Ohmachi and Masaki, 1964) *yezoemma* × *taiwanemma* (Ohmachi and Masaki, 1964)

Sex ratio:
+ normal.
± few male adults.
− no male adults.

Development:
+ normal as compared with the parents.
(+) accelerated.
± retarded but completed.
− retarded and not completed.

*Produced fertile hybrids.

Five classes may be recognized among the interspecific hybrids as to their degrees of developmental disturbance, as given in table 3. In the first class, the sex ratio was normal but the males developed unusually fast. In the second, about equal numbers of both sexes occurred, but the males required very long periods to mature. In the third, only a few males attained to the adult stage, and many were retarded and failed to mature. The males completely failed to mature in the next group of crossings, but the females were still normal in regard to their developmental rate. In the last, the females also suffered retarded development, though they were able to reach adulthood.

From this grouping, it would seem that there is a graded series of developmental disturbance, which might reflect the different degrees of genetic compatibility between the parental stocks. This seems to be partly, but not entirely, true. The correct portion is perhaps represented by the greater degrees of developmental failure in the hybrids in which *yezoemma* is involved. Careful examination of its morphological features, particularly of body conformation and male genitalia, suggests that this species is the most distinct in this group of *Teleogryllus*. Only this species frequently, though not always, lacks the mitre-like marking in the head.

Conjecture along this line must be stopped so far. Owing to the particular influence of sex-chromosomal genes, the reciprocal matings resulted in different degrees of developmental success. For example, both sexes of the hybrids between *mitratus* females and *taiwanemma* males showed the slightest degree of abnormality in their nymphal development, but the males of those from the reversed cross almost completely failed to mature. A decisive evidence against any attempt to relate the developmental success to the genetic similarity is found in the data on the fertility of hybrids. The progeny between *emma* females and *mitratus* males were least affected by hybridization in regard to their development, but they were entirely sterile. On the other hand, though the mating between *taiwanemma* females and *mitratus* males gave no male progeny, the female progeny were quite fecund and produced offspring which were also fertile.

A more weight should of course be laid on the fertility of hybrids in considering the genetic compatibility between the different forms, because this is an evidence of the successful meiosis and formation of viable gametes. There is then a possibility that the developmental failure or success in hybrids is not always related to the overall dissimilarity or similarity in the genetic make-up between the parents. The normal functioning of the endocrine system which governs development may be upset by a relatively slight degree of genetic defect. A subtle shift at any level of physiological functioning in the system may produce a profound influence. This is suggested by the possibility that the acceleration and retardation of development are effected by the same combination of parental species. That is, the same interspecific crossing produced, on the one hand, males which developed at an abnormally high rate and, on the other, those which were severely retarded and failed to mature for many months (*emma* ♀ × *taiwanemma* ♂ or *taiwanemma* ♀ × *mitratus* ♂). A threshold reaction may be involved in this case.

The above arguments indicate that it is not correct to infer the closeness between the parental stocks merely from the degrees of developmental success of hybrids. It is, however, almost certain that the three Japanese forms are different in their closeness to the tropical form, *mitratus*. Only *taiwanemma* produced fertile offspring when mated with *mitratus*, so that this form is more closely related to the tropical form than is either *emma* or *yezoemma*. Morphological comparisons support this. The three Japanese species can be distinguished from one another by the body conformation and the structure of male genitalia in most cases. By the same method, *emma* and *yezoemma* can be distinguished from *mitratus*. A difficulty arises only when *taiwanemma* is compared with *mitratus*. The two forms differ from one another in general body size and coloration, but their songs and male genitalia are very similar, if not identical.

The closest similarity of *taiwanemma* to *mitratus* may suggest that the two forms have been isolated from one another for a relatively short period. The greatest distinctness of *yezoemma* in this group of *Teleogryllus* may be a reflection of its longest isolation from the common ancestor. If these assumptions are incorporated into the hypothesis on their divergence in diapause behaviour and photoperiodic response, it may be claimed that the Japanese forms have been descended from the tropical ancestor in different geological times. It seems to be *yezoemma* which has diverged and invaded the area in the earliest time, this was perhaps followed by *emma*, and the northward extension of *mitratus* has recently reached the southern corner of this island chain and differentiated into *taiwanemma*. The last form may be still at an incipient state of speciation, and any proper taxonomic treatment may not be possible before the precise limits of its distribution and the whole range of geographic variation are made clear. The italicized name has been retained here, because there is as yet no enough reason for relegating it to synonymy.

The occurrence of repeated speciation from tropical populations and the subsequent multiple invasions do not contradict with the palaeoclimatic evidence, which indicates that there were episodic fluctuations of climatic conditions in the Pleistocene time (Minato *et al.*, 1965). The evolution of diapause behaviour and photoperiodic response would be most likely to occur, when populations which had previously no seasonal regulation mechanism encountered such climatic changes. When this occurred, the daughter population would survive the cooler climate *in situ*, while the non-diapause ancestral population would have to retreat southward and thus geographic isolation between them would be established. If such a process was repeated during multiple glaciations, there would be a group of related species in the temperate regions having a common ancestor in the tropics. The occurrence of groups of closely related species in various genera of the Gryllidae in different parts of the world may become comprehensible by this hypothesis.

Summary

Three Japanese forms of *Teleogryllus* are closely related to the tropical species, *T. mitratus*. They have, however, different means of seasonal regulation. The two northern species, *T. emma* and *T. yezoemma*, have obligatory diapause in the egg stage and belong to short-day insects in that their nymphal development is accelerated by a short photoperiod and delayed by a long one. On the other hand, the subtropical form, *T. taiwanemma*, is a long-day species, which enters a nymphal diapause in short-day conditons but not in long-day conditions. The tropical form, *T. mitratus*, is devoid of any conspicuous response to photoperiod in regard to its developmental rate.

All the Japanese forms produced hybrid offspring when mated with *T. mitratus*, and the hybrid eggs hatched in the periods almost comparable to the non-diapause egg stage. When the hybrid nymphs developed normally, the photoperiodic response of the parental forms was manifested in their deveopmental rate. The amount of this response was variable according to the combination of parental stocks. When the development of hybrids was not normal, the photoperiodic response was obscure.

The developmental disturbance was mainly observed in the male hybrids and grouped as follows: (1) the males developed at an unusually rapid rate and the females normally; (2) the males were retarded but completed development, and the females developed normally; (3) a few males were accelerated but the majority were retarded to develop and failed to mature, while the females developed normally; (4) the males were retarded to develop and died before maturity, and the females developed normally; and (5) the males failed to mature, and the females grew at an abnormally slow rate though they completed development.

The degree of developmental disturbance was not necessarily correlated with the overall genetic similarity or dissimilarity between the parental forms. Fertile hybrids were obtained only by the reciprocal matings between *T. mitratus* and *T. taiwanemma* even though the hybrid males (*taiwanemma* ♀ × *mitratus* ♂) were not viable. There was evidence of segregation in regard to the developmental disturbance in the F_2, F_3 or backcross generations.

Based on speculations about the divergence of diapause behaviour and photoperiodic response of these field crickets, it is surmised that each of the three Japanese species has independently descended from the non-diapause ancestor, *T. mitratus* itself or its predecessor, and a hypothetical picture on the speciation of the Japanese forms is presented. In this picture, *T. yezoemma* appears as a first offshoot from the tropical ancestor, *T. emma* comes next in the scene, and lastly *T. taiwanemma* is differentiated and reaches the island chain. It is pointed out that this repeated speciation and the subsequent multiple invasions can be accounted for by multiple glaciations.

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