4. Discussion

4-1. Ecological significance of initial diapause

This study has demonstrated that _M. hime_ and _C. bonneti_ are annual species and _M. japonica_ a plurennial species. One way to assess the ecological significance of initial diapause is a comparison of life cycles between annual and plurennial species. _M. japonica_ with initial diapause in addition to final diapause extends its distribution to a higher latitude and altitude than _M. hime_ and _C. bonneti_ without initial diapause. This fact may suggest that the occurrence of initial diapause at an early embryonic stage is related to some specific factor(s) associated with high latitudes and altitudes.

The ecological significance of initial diapause has been pointed out for species with a mixture of annual and biennial life cycles. Ando (1993a) showed that the proportion of eggs entering initial diapause was larger at higher latitudes in the Japanese _Gampsocleis_ species-complex. Ingrisch (1986b) reported that the induction of initial diapause was associated with parental short-day conditions in several species of European tettigoniids. Stick insect, _Didymuria violescens_ has annual and/or biennial life cycles because of two diapause stages in the embryogenesis (Readshaw & Bedford, 1971). Incidence of the first diapause was higher in the population from higher altitudinal areas. These facts may suggest that the occurrence of initial diapause is related to the latitudinal climatic gradient, most likely the amount of effective temperatures.
In Hirosaki, *M. hime* begins to oviposit earlier and produces eggs more intensively in a shorter time than *M. japonica* (Fig. 7). In *M. hime*, eggs laid in early September onwards in 1995 and those laid after mid August in 1997 could not reach the final diapause stage (Fig. 11). Eggs before this stage also can overwinter. However, eggs deposited late in the season might hardly have a chance of reproduction, because they hatch very late in the following year and thus have little time to reproduce after adult emergence (Fig. 12). Therefore, early and concentrated oviposition by *M. hime* is likely to be highly adaptive.

In *M. japonica*, oviposition occurs in mid August or later. Because this species enters initial diapause at the early stage of embryogenesis, eggs deposited in late summer and autumn may be able to attain the diapause stage for overwintering. Furthermore, those eggs that have overwintered in initial diapause start developing in the spring, but their development is interrupted again by the induction of final diapause. Thus, their hatching does not occur at least until the second spring. In fact, most eggs deposited in late August through mid October in 1995 hatched during a short period from late April to early May in 1997 (Fig. 13). This fact indicates that the relatively large variation in the time of oviposition does not affect the timing of hatching in the second spring. According to my unpublished observations, however, most eggs (91.2%; n=68) survived a 7-month exposure to 3°C even when this chilling treatment had been applied to newly deposited eggs (1-4 days old). This indicates the possibility that eggs deposited
late in the autumn can overwinter successfully even before reaching the initial diapause stage. If this is true, initial diapause would not be a prerequisite for overwintering.

On the other hand, some of the eggs deposited early in the season fail to maintain initial diapause until winter comes. As a result, they develop beyond this stage and overwinter (Table 4). Such individuals hatch after the first winter without entering final diapause. However, their hatching was apparently delayed compared to those which have overwintered at the final diapause stage (Fig. 14). A delay in hatching time results in a delay in adult emergence, and increases the risk of failure to reproduce. Thus, it seems to be important for eggs of *M. japonica* to pass the first winter in initial diapause so that they can postpone their hatching by entering final diapause until the second spring. In cold regions, hatching early in the season would be most important to attain sexual maturity early enough, and this appears to be made possible by passing the last winter in final diapause occurring when embryogenesis has been almost completed. Therefore, initial diapause may have a dual role: it prevents untimely hatching after the first winter, and ensures the successful induction of final diapause.

Variation in body size related to the geographic gradient of heat unit is known in several species of crickets (Masaki, 1996) and seems to be widespread among other insects. Body size of *M. japonica* is smaller at higher altitudes (Table 2). This reduced body size may be a result of adaptation to highlands where the growing season is short.
A prolonged initial diapause occurs in some eggs of *M. japonica* (Table 6), as in many other tettigoniid species (Ingrisch, 1986c; Hartley, 1990). Prolonged diapause occurs in many taxonomic groups and in different environmental conditions and has been the subject of several theoretical works (Takahashi, 1977; Walker, 1986; Seger & Brockmann, 1987). Takahashi (1977) theoretically showed that under unstable environmental conditions individuals that carry over to the next season, even when their number is not large, have a considerable effect on the population stability. Like the seed bank in desert annual plants (Cohen, 1966), prolonged diapause appears to be a bet-hedging tactic against unpredictable catastrophes. Ingrisch (1986c) showed that fecundity is not reduced by the prolonged initial diapause in *Decticus verrucivorus*. Moreover, he revealed that, in initial diapause eggs of *Eupholidoptera smyrnensis*, since the temperature dependence of oxygen consumption is low, the eggs need very little energy even at high temperatures (Ingrisch, 1987). Physiological costs of prolonged diapause are probably low in plurennial species of katydid because it occurs in a very early embryonic stage.

The presence of prolonged initial diapause in katydid species has been explained in relation to their unique abiotic factors. Ingrisch (1986c) and Hartley (1990) mentioned that developing embryos often encounter strong desiccation and that local summer fires sometimes sweep over an area killing all above ground stages. Ando (1993a) suggested the possibility that delayed adult emergence in unusually cool years decreases the chance of reproduction to *Gampsocleis*. 
species-complex. Therefore, prolonged diapause could be a bet-hedging strategy against unpredictable adversity caused by climatic conditions in katydids. The present results suggest that, besides air temperature, factors such as sunshine hours and snow cover period may greatly affect the life cycle of *M. japonica* at high altitudes.

*M. japonica* requires a high temperature for nymphal development and egg production (Fig.10, Table 3). It is well known that, in the sunlight, the internal body temperature of grasshoppers is higher than the air surrounding it as a result of basking (Uvarov, 1966, 1977; Begon, 1983). *M. japonica* frequently basks itself in the sun and the basking behavior is more common in highlands than in lowlands (unpublished observation). Perhaps, the blackish body color is also effective in absorbing radiant heat.

Results of field observations suggest that yearly fluctuations of weather conditions tend to affect the life cycle of the alpine populations more severely than that of the lowland ones (Fig.8). For example, the cool summer was probably the main factor that caused delayed adult emergence of *M. japonica* at the high altitudes in 1993. Moreover, short sunshine hours also seem to have contributed to the failure of this species to complete nymphal development and egg production at an alpine bog in Yamagata Pref. in 1995. Thus, the risk of extinction or a sharp reduction in population size would be higher in populations at higher altitudes. This leads to the prediction that the incidence of prolonged diapause is higher in populations at higher altitudes. This prediction may be supported by the results
of chilling experiment that initial diapause persisted longer in eggs derived from a higher altitude (Fig. 20, Table 6).

Thus, initial diapause in *M. japonica* may be an important life cycle trait in habitats where the growing season is short and unpredictable.

4-2. Mechanisms of initial diapause

Factors regulating the duration of initial diapause are another interesting problem. The mechanism of prolonged diapause has been poorly understood, though many examples of prolonged diapause have been reported (Danks, 1987). In most species, many individuals emerge after one year and fewer individuals remain in diapause for two or three years. However, in some species, a small fraction of individuals remains in diapause much longer (Barnes, 1952; Powell, 1989). Thus, the pattern of emergence from diapause is not always a steady decline over successive years. Prolonged diapause seems to be controlled mainly by genetic components, and partly by responses to environmental cues. Hedlin et al. (1982) confirmed that the incidence of prolonged diapause in the cone moth *Barbara colfaxiana* was greater at lower temperatures in the laboratory. Uhatinskaya (1972) reported that prolonged diapause of *Leptinotarsa decemlineata* occurred only in beetles in light sandy soils, but not in heavy, moist, clay soils. Ingrisch (1986c) showed that drought could serve as a token stimulus for the termination of prolonged initial diapause in *Tettigonia caudata*.

The means by which the difference among individuals are programmed have not been elucidated. In most species, a
difference in the intensity of diapause seems to be involved. In temperate regions, the seasonal temperature cycles such as cold-warm-cold-warm usually seem to be cues controlling prolonged diapause. There might be a mechanism for counting winters, and the number of winters required to terminate prolonged diapause might be considerably different among individuals. Whether such a mechanism really exist or not, the intensity of prolonged diapause seems to be gradually decreased with increasing number of cold-warm cycles.

In *M. japonica*, repetition of cold and warm treatments was effective to terminate the prolonged initial diapause. The three strains from different altitudinal areas showed similar patterns of response to high and low temperature cycles: cumulative percentage termination rapidly increased after the first chilling and thereafter slowly but increased again after the second chilling (Fig. 20). This fact suggests that in the field, the resumption of development synchronously occurs within a short time after overwintering but does not continue further until the next year. The percentage termination was significantly different among the three strains, and more eggs remained in initial diapause for a longer time in the population from the higher altitude.

The fact that three cycles required to reactivate all eggs in Series A and two cycles in Series B (Table 6) suggests that termination of initial diapause is affected not only by the number of chilling cycles, but also by the length of chilling. Dean and Hartley (1977) reported the results of chilling between 1 and 180 days in *Ehippigerida ephippiger* (formerly *Ephippiger cruciger*) and concluded that
prolongation of chilling for longer than 30 days did not increase the proportion of eggs resuming development. On the other hand, in *M. japonica*, the results of chilling between 15 and 720 days showed that percentage termination of initial diapause was clearly increased with prolonging chilling, though there was not significant difference among chilling ranging from 15 to 180 days (Fig. 21). However, the intensity of initial diapause was extremely high and variable among individuals.

Chilling for 680 days or longer seemed to cause many eggs to lose the ability of development. The low percentage termination in 680- and 720 day-treatments might be caused by increase of such eggs. Many eggs which had terminated the initial diapause after chilling for 520 days or shorter could hatch after the second chilling.

Though percentage termination tended to be higher after longer chilling, the maximum value of the percentage after chilling for 15 to 720 days was 89% in *M. japonica* (Ak965) in 1993 (Fig. 21). On the other hand, in the treatments with cold and warm cycles, percentage termination reached 100% after three cycles in Series A and two cycles in Series B (Table 6). In other words, all eggs resumed development after chilling for a total of 270 or 360 days in these treatments. Thus the initial diapause in *M. japonica* was terminated more smoothly by repetition of cold and warm treatments than by a single long cold treatment.

The results of constant temperature treatments showed that chilling is not always necessary to terminate the initial diapause (Fig. 17, Table 5). In general, diapause
Development in winter diapause is very slow at high temperatures whereas low temperatures allow rapid diapause development (Danks, 1987). However, the initial diapause in *M. japonica* was gradually terminated at constant temperatures between 15 and 25°C above the developmental zero and was terminated more effectively by 15 and 20°C. On the other hand, Hartley and Dean (1974) reported that in eggs of *Ehippigerida ephippiger* the highest proportion of initial development can be induced by starting incubation at 30°C. It is not certain whether the temperature during induction or after induction affects initial diapause intensity. For example, in the cricket *Teleogryllus emma* which has an annual life cycle with an embryonic diapause, temperature during inducing diapause influences the future intensity of diapause (Masaki, 1962).

The fact that chilling is not necessary to terminate initial diapause in all eggs suggests that initial diapause is similar to short diapause in many species that is sporadically terminated at a constant high temperature (Danks, 1987), but its intensity is much higher and variable. Rapid diapause termination at 20°C suggests possibility that eggs laid early in the season untimely develop beyond the initial diapause stage. Outdoor experiments showed that although *M. japonica* usually begins to deposit eggs in August or later (Fig. 7), there is a possibility that eggs unusually deposited in July hatch after the first winter (Table 4, Fig. 14). However, the initial diapause in this katydid seems to be obligate.

Different temperature treatments revealed the similar
and dissimilar characteristics of initial diapause compared to the common short winter diapause in other insects. Initial diapause was terminated at constant temperatures above the developmental zero and many individuals were reactivated by long chilling, but its intensity was very high and variable. Initial diapause eggs with higher intensity seem to be more likely to enter prolonged diapause. Cold and warm cycles terminated initial diapause more effectively than a single long chilling. Two successive exposures to cold and warm temperatures may be important cues for termination of this diapause.

4-3. Summer and final diapauses

According to the present study and my unpublished observation, summer and final diapauses occur in the all three species studied, while initial diapause only in M. japonica. These three types of diapause showed different thermal response to cope with different adaptational problems.

Developmental arrest in the egg stage at high temperature have been known in many species of katydids (Hartley, 1990) and at least one species of cricket (Tanaka, 1986). It generally occurs at an intermediate stage of embryonic development before substantial water absorption. In this thesis, I conveniently use "summer diapause" for the developmental arrest. However, Hartley (1990) refers such arrest to the high temperature inhibition perhaps caused by lowering of developmental temperature range with age. Ingrisch (1987) suggests that, in Eupholidoptera smyrnensis,
it is energetically profitable to the embryo to spend the hot season in penultimate diapause (at stage 20). Though developmental delay and summer diapause was observed under high temperature conditions in the three species used for the present study, it must not occur in *M. japonica* and northern populations of *M. hime* and *C. bonneti* in their original habitats where summer is rather cool. On the other hand, the eggs of southern populations of the latter two species must encounter high temperatures. In *M. hime*, because Os strain begins to oviposit in late June, the eggs of it must spend the hottest season (Fig. 30). In outdoor experiment, the embryonic development of Os delayed more conspicuously in the eggs deposited earlier, while the eggs of Ao developed without any delay irrespective of the oviposition time (Fig. 31). It seems that the developmental delay and summer diapause allow the eggs to survive the hottest season and to reach the final diapause stage more synchronously in autumn than expected from the long egg-laying period.

Final diapause occurring in the mature embryo is never terminated and the eggs gradually shrink and eventually die if they are kept at 25°C or higher. It can be characterized by the requirement for a low temperature for termination as in other pharate larval diapause (Hodson and Weinmann, 1945; Masaki, 1956; Readshaw and Bedford, 1971). Intra- and interspecific variation of final-diapause intensity was found in the present study (Fig. 15, Fig. 27). Diapause intensity is higher in plurennial species (*M. japonica*) than in annual species (*M. hime*), and also in the population from the southern region. These results may be explained as follows.
In the plurennial (and biennial) species mainly inhabiting cool regions, the eggs which terminated initial diapause in winter resume development soon after the soil temperature rises above the lower threshold and reach the final diapause stage without delay. Therefore, they have to spend under mild temperature conditions as 15-20°C for a long time. In annual species, the eggs of southern population must spend long autumn after termination of summer diapause.

Ingrisch (1985) suggests another role of final diapause in life cycle regulation. He shows that there are two patterns of hatching in tettigoniid species. In late hatching species (e.g. Conocephalus, Phaneroptera), the hatching date is not affected by the length of cooling period, but in early hatching species (e.g. Metrioptera, Tettigonia), extension of the cooling period advances the hatching date. He considered this response to be an adaptation to hatching as early as late spring. In M. hime (Ao140), the mean hatching time was decreased by prolongation of chilling to 100 days, but further prolongation did not affect the hatching time (Fig. 16). In M. japonica (Ao220), on the other hand, the mean hatching time was shortened by prolongation of chilling to 180 days.

Final diapause may be more important as regulator for favorable timing of hatch in M. japonica mainly inhabiting snowy areas where the growing season is short.

Some species of egg-overwinterer are known to be cold hardy and able to spend winter safely in the pre-diapause stage (Tanaka, 1992; Ando, 1993b; Ishiguri, 1997). Especially, most individuals overwinter at pre-diapause
stages in the northern populations of the rice grasshopper, *Oxya yezoensis* (Ando, 1993b) and the katydid, *Conocephalus japonicus* (Ishiguri, 1997). Ishiguri pointed out that further study is necessary to find other examples of overwintering at the pre-diapause stage. Eggs of *M. hime*(A0140) before the final diapause stage also could overwinter and hatched after the first winter without entering final diapause (Fig. 12). However, because those eggs hatched much later in the season, overwintering before final diapause seems not to be adaptive.

In univoltine egg-overwinterers of some crickets (Masaki, 1996) and at least one grasshopper (Ando, 1993b), photoperiod has been known to control the rate of larval development. Short photoperiod accelerates, and long photoperiod decelerates, larval development. Moreover, larval development time tends to be shorter in northern populations than in southern population. As a result, both northern and southern populations of those species emerge as adults at almost the same time in autumn and can lay eggs in appropriate time before winter (Masaki, 1963; Ando, 1998). Adjustment of adult emergence time to autumn is important for these species to prevent untimely diapause termination before winter, because intensity of their egg diapause is relatively low and can hatch under constant high temperature conditions.

In *M. hime*, on the other hand, adult emergence occurred about one month earlier in the southern population than in the northern population in their original habitats and regulation mechanism of nymphal development is unknown. As a result, in the southern population, eggs began to be laid
before mid summer. Their development was delayed and they entered summer diapause at stages 18-19 in the hottest season. After summer, they synchronously resumed development and reached at the final diapause stage. The higher diapause intensity of the southern eggs is useful to survive the long mild temperature conditions.

The role of egg stage in life cycle regulation is very important in the biennial and plurennial species. However, this stage also plays an important role in annual species.
5. Summary

The life cycle and diapause characteristics were compared among three katydid species to understand the ecological function of the egg stage with two or three diapause stages. *Metrioptera japonica* inhabiting northern lowlands and southern alpine regions required two or more years to complete its life cycle and showed two types of embryonic diapause: one occurs just after blastoderm formation (initial diapause) and the other shortly before hatching (final diapause). Some eggs of this species remained in initial diapause for several years. The incidence of prolonged diapause was higher in eggs derived from higher altitudes. *Metrioptera hime* and *Chizuella bonneti* are univoltine and entered diapause only at a late embryonic stage. These univoltine species hatched over a relatively long period of time in spring but emerged as adults by summer, because they occur only at lowlands. The time of adult emergence in *M. japonica* at high altitudes varied greatly from year to year, and adults appeared relatively late in the season. Because eggs of this species survived a period of chilling even before reaching the initial diapause stage, initial diapause is probably not a prerequisite for overwintering. Variation in the time of oviposition or induction of initial diapause did not affect the timing of hatching that occurred two years later. It appears that in *M. japonica* initial diapause plays an important role in the control of the life cycle in habitats where the growing season is short and unpredictable.
In *M. hime*, the embryo developed without delay before reaching stage 24 at 20, 22.5 and 25°C. At 27.5 and 30°C, on the other hand, marked delay was observed, although the initial development proceeded quickly. This retardation at high temperatures was more striking in the southern population. Especially, at 30°C, eggs stopped development at stages 18-19 and were considered to enter summer diapause, during which water absorption was suppressed. A shift of temperature from 30°C to 20 and 25°C terminated summer diapause and the eggs started to develop and absorb water rapidly, while the eggs remaining at 30°C stayed at stages 18-19. Adult emergence occurred in early-mid June and began to lay eggs before mid summer. In an outdoor experiment, embryonic development delayed more conspicuously in the eggs deposited earlier in a southern population. It seems that the developmental delay and summer diapause allow the eggs to survive the hottest season and to reach the final diapause stage more synchronously in autumn than expected from the long egg-laying period.

Final diapause occurring in the mature embryo is never terminated and the eggs gradually shrink and eventually die if they are kept at high temperatures. A cold treatment is necessary to terminate final diapause. Diapause intensity is higher in the plurennial species (*M. japonica*) than in the annual species (*M. hime*), and also in the population from the southern region. The high intensity of final diapause enables eggs to survive long period under mild temperature conditions before winter.
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