学位論文内容の要旨

博士の専攻分野名称：博士（農学）　　　　　　　氏　名：Nousheen Parven

学位論文題名

Effects of high temperature stress on aphid growth and reproduction: responses of clones with diverse genetic backgrounds and aphid symbionts to heat stress

（高温ストレスがアブラムシの成長と繁殖に与える影響：多様な遺伝的背景を持つクローンおよびアブラムシ共生生物の熱ストレスに対する反応）

According to global warming scenarios, it is estimated that the frequency and intensity of high temperature events during summer will increase in the future. In this study, I firstly investigated the effects of high temperature stress on the growth and reproduction of aphids by selecting clones with various genetic backgrounds. Aphids are major pests causing damages to crops and trees, and thus it is important to evaluate the effects of high temperature stress from the viewpoint of pest management. In addition, aphids harbor obligate intracellular endosymbiont *Buchnera*, and symbiosis with *Buchnera* has a critical effect on aphid survival and reproduction. As a second objective, I evaluated the copy numbers of genes specific to *Buchnera* and host aphids after heat shock to analyze how high temperature stress affects the growth of both *Buchnera* and the host aphids.

In Chapter 1, I investigated the relationships among aphid clones, high temperature treatments, and temperature acclimations. Two clones of *Acyrthosiphon pisum*, collected in Sapporo and Tokyo, and three clones of *Megoura crassicauda,* collected in Sapporo, Tokyo and Nagasaki, were used for experiments. Newly hatched nymphs were treated with a single heat shock (35°C for 6 h), repetitive heat shock (35°C for 6 h for 3 consecutive days), or a control (constant 20°C). Before the heat shock treatment, the clones were reared for four or more generations at constant 20°C, constant 22°C, or increasing temperature from 22°C to 24°C in order to investigate the possibility of acquiring high temperature tolerance (acclimation effects). The Sapporo clone was more susceptible to the high temperature treatment than the Tokyo clone in *A. pisum*, and both survival rate and the number of offspring were significantly reduced in the repetitive heat shock treatment. *M. crassicauda* was more tolerant to heat shock treatments than *A. pisum*. Especially in the Tokyo and Nagasaki clones, no significant decrease in survival was observed. The latitudes of the collection sites were related to the thermotolerant ability of *A. pisum* and *M. crassicauda* clones. Analysis of the relationship between the pretreatments and the heat shock treatments showed that rearing at 22°C for four generations mitigated the effects of heat shock only in the Sapporo clone of *A. pisum*. This result suggests that the acclimation effect may be an adaptation in aphids inhabiting low temperature regions. However, when the temperature was increased from 22°C to 24°C for six generations to promote acclimatization, there was a significant decrease in the number of offspring compared to the control in *A. pisum*. In addition, no recovery in fecundity was observed in *M. crassicauda*. These results indicate that the aphids of both species are unlikely to acquire high temperature tolerance through acclimatization, and that the population will be more damaged by continued higher temperatures in summer.

In Chapter 2, I investigated the relationship between inbreeding/outbreeding and heat shock treatments. In aphids, the possibility of intra-clonal mating is high, and this may result in the production of clones of the selfing origin. Two clones were selected as parents, and the sexual generation was induced under a low temperature and short-day conditions to obtain eggs. New clones were established from nymphs hatching from the overwintered eggs, and two sets of the parental clone–selfed progeny clone were prepared. These clonal pairs were subjected to one of the single heat shock treatment, the repeated high temperature treatment, and the control treatment (constant 20°C). The results showed that there was no significant difference in the survival rate and the number of offspring between the parent and the selfed progeny at 20°C. However, the survival rate and the number of offspring of the selfed progeny were significantly lower than those of the parent clones under the repeated heat shock treatment. These results demonstrate for the first time in aphids that the progeny of the selfing origin are likely to be driven to extinction due to pronounced inbreeding depression under high temperature stress, even if their reproductive performance is comparable to that of the parent clone under benign conditions.

In Chapter 3, the effects of heat shock on the cell proliferation of the host aphid and the symbiont *Buchnera* were investigated. When the aphids reached the fourth instar stage after heat shock, the gene copy numbers of the host aphid and *Buchnera* were investigated. Quantitative PCR was used to count the copy number (density) of the aphid nuclear gene (elongation factor 1-alpha) and the copy number (density) of the *Buchnera* 16S-rRNA gene in the current and next generation after the heat shock treatment. Aphid body length, aphid nuclear gene density and *Buchnera* gene density were all negatively affected by heat shock treatment. When statistically evaluated at a constant body length, aphids treated with heat shock at the first instar showed lower *Buchnera* gene density and lower aphid gene density than aphids in the control. When the relationship between *Buchnera* gene density and aphid gene density was represented on the log-log axis, it was clear that *Buchnera* gene density increased with aphid gene density, but showed a negative allometry to aphid gene density. Compared with the control aphids, aphids subjected to heat shock showed relatively lower *Buchnera* gene density to aphid gene density. In addition, heat-shock treated aphids were often sterile even when they reached adulthood. These sterile adults retain very low *Buchnera* gene densities. Aphids that are subjected to the single heat shock treatment regained *Buchnera* gene density in their offspring generation. However, aphids subjected to the repeated heat shock treatment showed significantly lower *Buchnera* and aphid gene densities. In conclusion, high temperature stress inhibited cell proliferation of aphids and *Buchnera* in both the current and the next generation of treated aphids, but the negative effects were more pronounced in *Buchnera*. Since *Buchnera* provides essential amino acids, vitamins, and proteins to the host aphids, continued high temperature stress under a global warming scenario may reduce aphid growth and reproduction, possibly leading to the extinction of local aphid populations.

In summary, this paper provides a multifaceted analysis of the effects of high temperature stress on aphids and provides important insights into the increase or decrease in local aphid populations under a global warming scenario.