A shift toward early reproduction in an introduced herbivorous ladybird

T A K A Y U K I   O H G U S H I [1] and H I R O I C H I   S A W A D A [2] Laboratory of Entomology, Faculty of Agriculture, Kyoto University, Japan

Abstract. 1. In May 1971, fifteen male and thirty female overwintering adults of a thistle-feeding ladybird *Epilachna niponica* were taken from the Asiu Experimental Forest of Kyoto University and introduced into the Botanical Garden of Kyoto University, 30 km south of Asiu and 10 km south of the southern limits of its distribution. The introduced population established successfully and thereafter maintained densities sufficiently high to defoliate their host plants.

2. Reproductive parameters (reproductive lifespan of females, overall fecundity, and oviposition schedules) of the introduced population were compared with those of the source population 10 years after the introduction. Comparisons were made in the laboratory under five temperature regimes.

3. The lifespan of females differed significantly with temperature, decreasing linearly with increasing temperature. Temperature also had a significant effect on overall fecundity for both populations; overall fecundity at 15 and 30 °C was significantly lower than that at the 20–25 °C regimes.

4. The oviposition activity of the introduced population was significantly higher than that of the source population at 23 and 25 °C. The lifespan of females of the introduced population was also significantly shorter than those of the source population at 20 and 25 °C.

5. The reduced lifespan and higher oviposition activity of introduced females indicate that they directed greater reproductive efforts early in their reproductive lifetime than those of the source population.

Key words. Early reproduction, *Epilachna niponica*, fecundity, introduction experiment, life-history evolution, natural selection, oviposition rate, reproductive lifespan, source population, temperature.

Introduction

Monitoring the life-history traits of organisms following their colonization of new areas is of paramount importance for an understanding of the life-history evolution of colonizing species (MacArthur & Wilson, 1967; Endler, 1986). In this context, artificial introduction experiments are unique manipulations to test how life-history traits of the colonizing species are moulded by natural selection in new habitats (Endler, 1980; Stearns,

1983; Reznick & Bryga, 1987; Reznick *et al.,* 1990). Although several authors have considered the conditions necessary for the successful introduction of herbivorous insects as agents of biological control for weeds (Crawley, 1987; Myers, 1987; McEvoy *et al.,* 1991), surprisingly few studies have compared the reproductive traits of the introduced population with those of the source population of insect herbivores.

Epilachna niponica is a univoltine herbivorous ladybird that feeds exclusively on leaves of thistle plants. Having studied a natural population of the ladybird in the Asiu Experimental Forest of Kyoto University in the 3-year period 1974–76, Nakamura & Ohgushi (1979, 1981) showed that the beetle population persisted at relatively low densities and there was no evidence of host plant defoliation. In May 1971, fifteen males and thirty females of overwintering adults collected from the Asiu population were introduced into the Botanical Garden of Kyoto University, 30 km south of Asiu and 10 km south of

Correspondence: Dr Takayuki Ohgushi, Institute of Low Temperature Science, Hokkaido University, Sapporo 060, Japan. E-mail ohgushi@orange.lowtem.hokudai.ac.jp

[1]Present address: Institute of Low Temperature Science, Hokkaido University, Sapporo 060, Japan.

[2]Present address: Faculty of Agriculture, Shiga Prefectural Junior College, Kusatsu, Shiga 525, Japan.

the southern limits of its distribution. The introduced population established successfully and in 1974 thistle plants were observed to be highly defoliated by the ladybird. Thereafter, thistle plants at the Botanical Garden have been severely damaged by larval feeding every year.

Investigations on the introduced population began in the spring of 1975, 4 years after its introduction (Sawada & Ohgushi, 1994; Ohgushi & Sawada, 1995). Ohgushi & Sawada (1995) compared the demography of the introduced population with that of the source population and suggested that, coupled with a lower level of immature mortality, a twofold increase in the mean fecundity of the introduced population contributed to higher population density at adult emergence, leading to severe host plant defoliation in the Botanical Garden. Furthermore, fecundity tended to increase over the seven-year study period (Ohgushi & Sawada, 1995). However, fecundity estimated in the field may be affected greatly by environmental conditions and/or adult densities. For example, the 3–5 °C higher temperatures at the Botanical Garden may have caused the higher fecundity relative to the Asiu population. Also, for the introduced population, oviposition schedules may be altered by the early onset of oviposition, which occurred 1 month earlier than at the source population (Sawada, 1984). Thus, a comparison of these oviposition attributes of the two populations under identical laboratory conditions is necessary to determine whether the introduced population has changed in terms of not only reproductive parameters but also population dynamics through changes in the reproductive parameters after introduction.

In 1981, 10 years after introduction, a laboratory experiment to compare oviposition parameters of individual females (reproductive lifespan, fecundity and temporal oviposition activity) of the introduced and source populations was conducted to determine whether these parameters of the ladybird changed after introduction.

Materials and Methods

The laboratory experiment to compare realized fecundity, lifespan of reproductive females, and temporal oviposition activity of the introduced and source populations was conducted at five temperatures: 15, 20, 23, 25 and 30 °C.

In 1981, 10 years after introduction, fifty pairs of over-wintered adults were collected from each of the introduced and the source populations, when they had just emerged but had not yet begun oviposition. Each pair of reproductive adults was placed in a separate plastic cup (9 cm in diameter and 4 cm in depth) with a plastic lid. They were classified into five groups, each consisting of ten pairs from each population, and were kept in controlled-environment chambers at 15, 20, 23, 25 or 30 °C under a 16L: 8D photoperiod. Ample amounts of fresh leaves of the thistle *Cirsium nipponicum,* grown in the Botanical Garden, were offered every 2 days throughout the experiment. For each pair, the number of eggs laid was recorded every 2 days until the death of the female. The experiment lasted 60 days for both populations. Because the introduced population began oviposition 1 month earlier than the source

population (Sawada, 1984) and overwintered adults of the source population emerged in early May (Nakamura & Ohgushi, 1979), the experiment started on 15 April for the introduced population and on 15 May for the source population, corresponding with the commencement of oviposition in the field. Ohgushi (1986) showed that leaf amino acid and water contents of the thistle *C. kagamontanum* tended to decline throughout the season, and that the seasonal decline in leaf quality was enhanced by leaf damage. However, young leaves without damage showed no differences in water and amino acid contents up to August. Thus, young intact leaves were used to remove the effects of seasonal changes in host quality on oviposition attributes between the two populations.

Two-way analysis of variance was used to assess the effects of temperature and population on overall fecundity and female lifespan during the experimental period. The effects of population and period on oviposition rate (the number of eggs laid per female per 2 days) were also determined by two-way analysis of variance in each 10-day period during the experiment. Scheffe's comparison of means was performed to determine whether mean fecundity, lifespan and oviposition rate were significantly different in terms of temperature, population or period.

Results

Reproductive lifespan

The lifespan of reproductive females differed significantly at different temperatures, decreasing linearly with increasing temperature (Table 1, Fig. 1). In particular, all females at 30 °C in both populations died before the end of the experiment (see Fig. 3). Significant differences were also found between the two populations at 20 and 25 °C; the lifespan of the introduced population was approximately 15 days shorter than that of the source population (Fig. 1, $P < 0.05$).
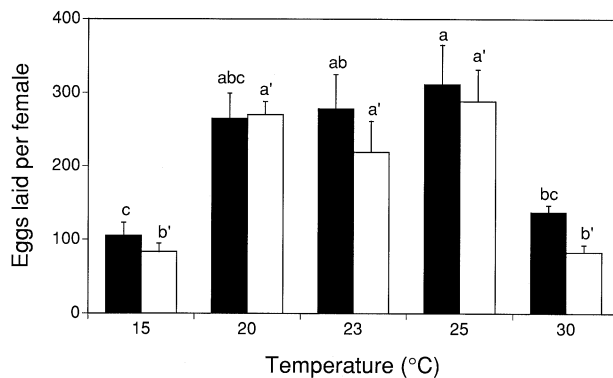
Overall fecundity

Temperature had a significant effect on overall fecundity (eggs laid per female during a 60-day period) in both populations (Table 1, Fig. 2). Reproductive females reared at 15 and 30 °C laid significantly fewer eggs over the experimental period than females in the intermediate temperature regimes. Mean fecundity of the introduced population was higher than that of the source population at each temperature except 20 °C, but the differences were not statistically significant (Table 1, Fig. 2).

Temporal patterns of oviposition activity

Oviposition rate (the number of eggs laid per female per 2 days) was higher at 23 and 25 °C in both populations (Fig. 3) and lowest at 15 and 30 °C. In addition, all females at 30 °C died by day 40 and day 50 for the introduced and source

Table 1. Analysis of variance for effects of temperature and population on reproductive lifespan and overall fecundity of females.

Source	d.f.	Reproductive lifespan			Overall fecundity		
		MS	F	P	MS	F	P
Population	1	1600.00	8.413	0.0047	23531.56	2.187	0.1427
Temperature	4	1868.44	9.825	< 0.0001	180624.29	16.786	< 0.0001
Population × temperature	4	272.00	1.430	0.2304	3529.59	0.328	0.8585
Error	90	190.18			10760.58		
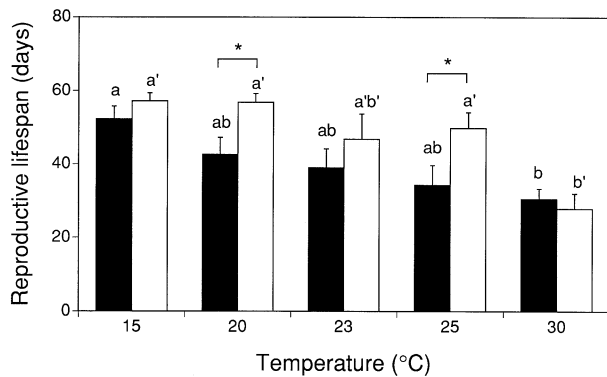
Fig. 1. Reproductive lifespan of females from the introduced population (■) and the source population (□). Means and 1 SE are presented. Means with different letters are significantly different among temperatures (Scheffe's test: $P < 0.05$). Asterisks show significant differences between the introduced and source populations (Scheffe's test: $P < 0.05$).

Fig. 2. Overall fecundity in the experimental period (60 days) of females from the introduced population (■) and the source population (□). Means and 1 SE are presented. Means with different letters are significantly different among temperatures (Scheffe's test: $P < 0.05$).

populations, respectively. In every temperature regime except 30 °C, the number of reproductive females alive declined faster from day 20 to day 40 in the introduced population than in the source population. Survival rate of females at the end of the experiment in the introduced population was significantly lower than that in the source population at 20 and 23 °C (G-test with the Williams' correction; $G_{adj} = 4.93$, d.f. $= 1$, $P < 0.05$).

Comparison of the mean oviposition rate in each 10-day period shows that the introduced population generally had a higher oviposition rate than the source population, although the difference was only significant at 23 and 25 °C (Table 2). The increased oviposition rate of the introduced population was particularly evident early in reproductive life at 23 and 25 °C (Fig. 4).

Discussion

This study demonstrated that there were significant effects of temperature on reproductive parameters of *E. niponica*, and that, even when the temperature effects were accounted for, there remained some significant differences in female lifespan and oviposition rate between the introduced and source populations.

In a physiological sense, there is an optimum temperature for insect species to produce eggs in ovaries, outside which fecundity and oviposition rate gradually decrease. Several laboratory studies of herbivorous insects have shown that beyond the optimum temperature fecundity and oviposition rate are reduced (e.g. Greenfield & Karandinos, 1976; Dingle *et al.,* 1982; Baldwin & Dingle, 1986; Minkenberg & Helderman, 1990; Carroll & Quiring, 1993). For *E. niponica,* overall fecundity reached around 250 eggs at 20–25 °C in both populations (Fig. 2). In the field, the reproductive period of both populations coincides with this favourable temperature regime for oviposition (Sawada & Ohgushi, 1994). Likewise, the temperature significantly affected the longevity of reproductive females; lifespan consistently decreased with increasing temperature (Fig. 1). A reduced lifespan of reproductive females with increasing temperature has also been reported in the milkweed bug (Dingle *et al.,* 1982; Baldwin & Dingle, 1986), the lesser peach tree borer (Greenfield & Karandinos, 1976), and the tomato leaf-mining fly (Minkenberg & Helderman, 1990).

There were no significant differences in overall fecundity between the two populations at any temperature. Because of a 3–5 °C higher temperature at the Botanical Garden than at the Asiu Experimental Forest (Sawada & Ohgushi, 1994), it is possible that the higher temperatures may have generated the increased fecundity observed previously in the introduced population (Ohgushi & Sawada, 1995). This is, however, unlikely because realized fecundity did not differ significantly between the two populations in any pairwise comparisons in
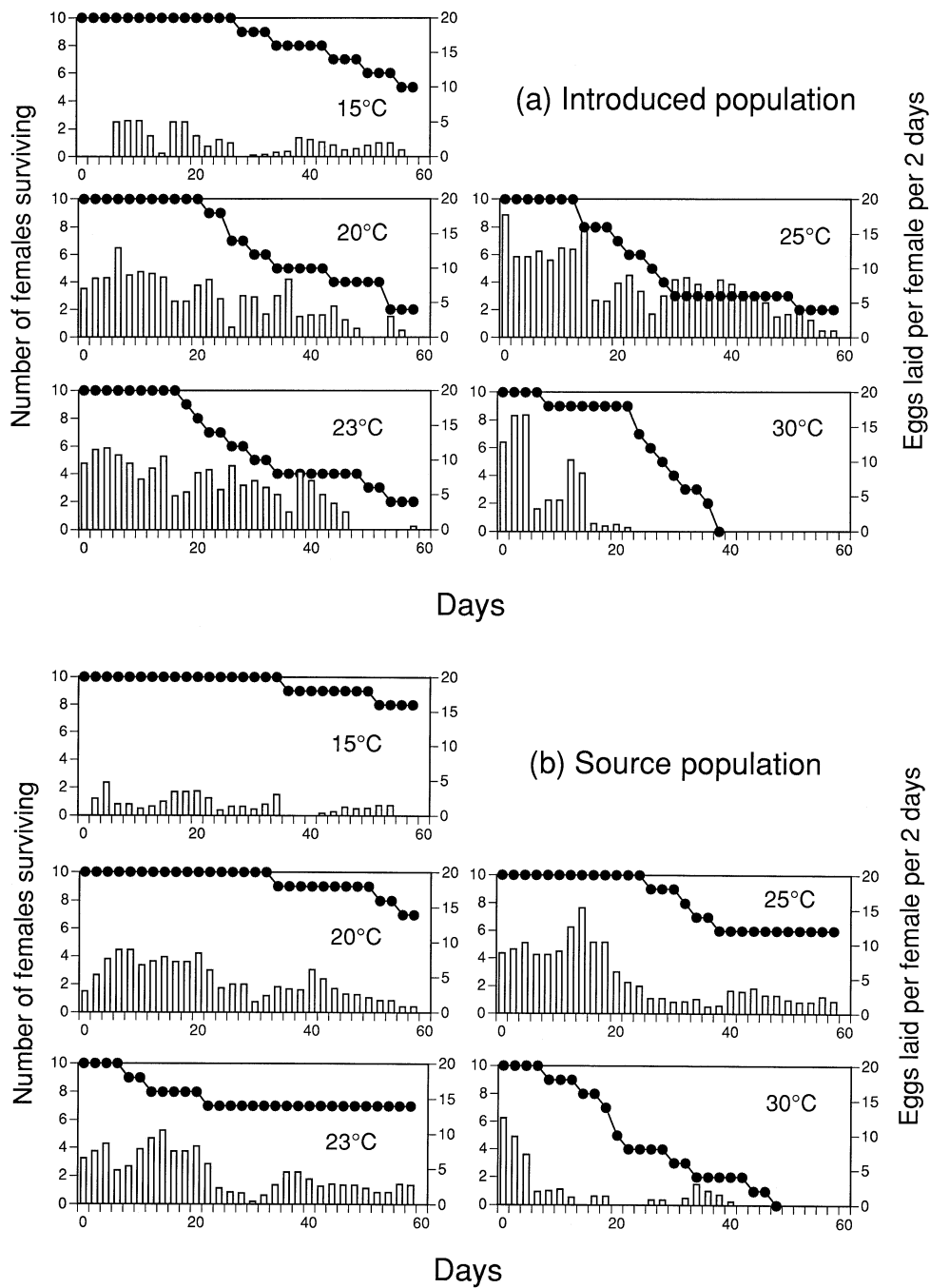
Fig. 3. Number of females surviving (●) and oviposition rate (number of eggs laid per female per 2 days) (□) during the experimental period at five different temperatures in (a) the introduced population and (b) the source population.

the 20–25 °C regimes (Fig. 2). Hence, the difference in potential fecundity between the two populations cannot explain the increased number of eggs laid at the Botanical Garden. In contrast to the highly confined introduced population, some reproductive females in the source population are likely to emigrate from the study site for oviposition, moving to nearby individuals of the continuously distributed host plants. This

may account for the reduced mean realized fecundity at the study site in Asiu (Nakamura & Ohgushi, 1983).

Perhaps more interesting is the fact that some reproductive parameters have changed since introduction. Even when the temperature effects were removed, females from the introduced population had a significantly shorter lifespan and higher oviposition rate than females from the source population

Table 2. Analysis of variance for effects of population and period on oviposition rate in each 10-day period over the experimental period at five temperature regimes.

Source	d.f.	MS	F	P
15 °C				
Population	1	3.36	1.73	0.1941
Period	5	5.84	3.01	0.0191
Population × period	5	1.20	0.62	0.6836
Error	48	1.93		
20 °C				
Population	1	7.37	2.26	0.1394
Period	5	66.37	20.34	< 0.0001
Population × period	5	5.61	1.72	0.1478
Error	48	3.26		
23 °C				
Population	1	27.65	8.81	0.0047
Period	5	79.76	25.41	< 0.0001
Population × period	5	17.30	5.51	0.0004
Error	48	3.13		
25 °C				
Population	1	92.71	22.11	< 0.0001
Period	5	137.93	32.90	< 0.0001
Population × period	5	17.15	4.09	0.0036
Error	48	4.19		
30 °C				
Population	1	24.70	2.47	0.1253
Period	4	114.64	11.46	< 0.0001
Population × period	3	18.32	1.83	0.1599
Error	34	10.00		

under temperature regimes that the two populations actually experience in the field. Note that the between-population difference in reproductive lifespan may be somewhat underestimated, because longevity of individuals alive at the end of the experiment (day 60) was under-estimated. The source population had a larger proportion of long-lived females that survived over 60 days than the introduced population (Fig. 3). The higher oviposition rate probably compensated for the shorter lifespan of females in the introduced population, resulting in no significant differences in total fecundity between the two populations (Fig. 2). Overall, because of the reduced lifespan and increased oviposition rate of females, the introduced population directed more resources to reproduction in the early part of the reproductive period than the source population. One could argue that female size might affect the reproductive parameters, resulting in the differences observed between the two populations, however, this is unlikely because fecundity and reproductive lifespan are totally independent of female size (Ohgushi, 1996).

What are the ecological and evolutionary implications of the shift toward early reproduction in the introduced population? Because natural selection favours individuals with higher fitness, the different pattern of oviposition activity may be explained by selection for early reproduction in the introduced

population. The introduced population had frequently encountered host-plant depletion by larval feeding due to increased population density. A number of thistle plants in the Botanical Garden were completely defoliated in late June in 1974, 1975, 1977, 1979 and 1981, and a great number of larvae that hatched late in the season died from starvation (Sawada, 1984; Ohgushi & Sawada, 1995). In addition, there was no season-specific mortality that reduced the survival of individuals born early in the season. Thus, because intraspecific competition for a limited food source increases during the season, later-hatched larvae have lower fitness than early hatched larvae. In comparison, because of greater mortality during the immature stage, the source population rarely reached high enough densities to cause food depletion (Nakamura & Ohgushi, 1981). Also, arthropod predation, a main cause of larval mortality, operated early in the reproductive season, thereby reducing egg and larval survival of early cohorts in the source population. This implies that an adult female of the introduced population should lay eggs early in the season, to increase its lifetime reproductive success. In contrast, early reproduction is unlikely to improve a female's reproductive success in the source population. The temporal correlation of enhanced oviposition activity with higher performance of offspring of early cohorts in the introduced population suggests that early reproduction has been moulded by natural selection since introduction. In this context, another hypothesis is that the shift toward early reproduction is not due to the selective force (lower level of resource availability for later cohorts) associated with the Botanical Garden, but due to gene flow from neighbourhood populations. However, this is unlikely. The Botanical Garden is located 10 km south of the southern limits of the distribution of *E. niponica*. Furthermore, the ladybird has an extremely limited dispersal ability; mean distance travelled throughout its lifetime is less than 10 m for both sexes (Ohgushi, 1983; Sawada, 1984), suggesting that 10 km is a sufficient distance for population isolation. Hence, it is most likely that the introduced population has been genetically separated from any other *E. niponica* population since introduction.

Having conducted an experimental study on oviposition schedules of two local populations (A and F) of *E. niponica*, which are 10 and 20 km east of the Asiu Experimental Forest, respectively, Ohgushi (1991) provided strong evidence to support the hypothesis that early reproduction of the introduced population had been favoured by natural selection. He found that population A reproduced significantly earlier, with a shorter reproductive lifespan of females, while population F delayed reproduction with a longer lifespan. These populations were only 10 km apart. Moreover, there was a strong correlation between reproductive activity and offspring lifetime fitness of each cohort throughout the reproductive season in each population. This indicates that there is a strong linkage between temporal patterns of oviposition activity and offspring fitness of the ladybird. The laboratory experiment suggested that the differences in oviposition schedules and lifespan of females between populations A and F had a genetic basis (Ohgushi, 1991).

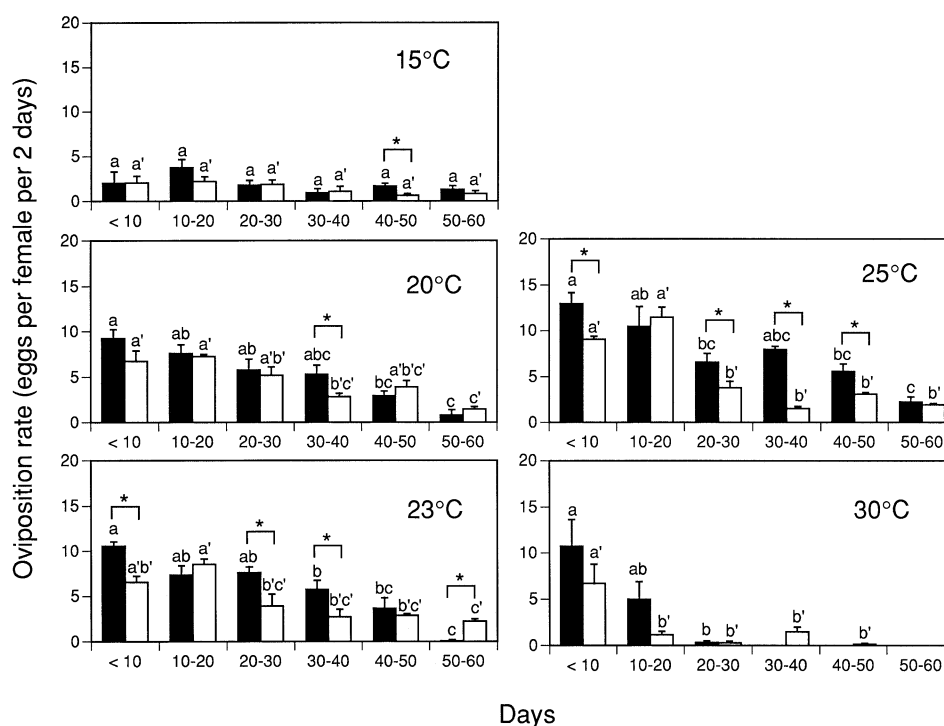No introduction experiments have been carried out for

Fig. 4. Oviposition rate (number of eggs laid per female per 2 days) in each 10-day period over the experimental period at five temperature regimes for females from the introduced population (■) and the source population (□). Means and 1SE are presented. Means with different letters are significantly different among periods (Scheffe's test: $P < 0.05$). Asterisks show significant differences between the introduced and source populations (Scheffe's test: $P < 0.05$).

herbivorous insects to evaluate how life-history parameters are altered by natural selection. In his excellent introduction experiment, Endler (1980) illustrated that colour patterns of guppies represent a balance between selection for crypsis by predators and selection for conspicuousness by sexual selection. Changes in colour patterns occurred within fifteen generations after introduction, responding to selection by predation. Following this experiment, Reznick *et al.* (1990) continued long-term introduction experiments with differential predation on natural populations of guppies over 11 years, and found that there were significant changes in some reproductive parameters, including size and age of females at reproduction, brood size, and fecundity, that corresponded to theoretical predictions on life-history evolution. Laboratory experiments of artificial selection on reproductive parameters of *Drosophila* have also shown that temporal oviposition pattern is genetically determined, and early and delayed reproduction can easily be modified by selection (Rose & Charlesworth, 1981; Rose, 1984, 1991; Luckinbill *et al.*, 1984).

Although there is a strong likelihood that decreasing food availability for larvae during the season at the Botanical Garden has selected for early reproduction, it is not possible to rule out an alternative hypothesis that genetic drift may have caused the shift in oviposition pattern, because a genetic comparison of the introduced and source populations was not conducted. Thus, further investigation of the genetic structure of the introduced population is needed to determine the mechanism

responsible for the early reproduction of the introduced population.

References

Baldwin, J.D. & Dingle, H. (1986) Geographic variation in the effects of temperature on life-history traits in the large milkweed bug *Oncopeltus fasciatus. Oecologia*, **69**, 64–71.

Carroll, A.L. & Quiring, D.T. (1993) Interactions between size and temperature influence fecundity and longevity of a tortricid moth, *Zeiraphera canadensis. Oecologia*, **93**, 233–241.

Crawley, M.J. (1987) What makes a community invasible?

Colonization, Succession and Stability (ed. by A.J. Gray, M. J. Crawley and P. J. Edwards), pp. 429–453. Blackwell Scientific Publications, Oxford.

Dingle, H., Blau, W.S., Brown, C.K. & Hegmann, J.P. (1982) Population crosses and the genetic structure of milkweed bug life histories. *Evolution and Genetics of Life Histories* (ed. by H. Dingle and J. P. Hegmann), pp. 209–229. Springer Verlag, New York.

Endler, J.A. (1980) Natural selection on color patterns in *Poecilia reticulata. Evolution*, **34**, 76–91.

Endler, J.A. (1986) *Natural Selection in the Wild.* Princeton University Press, Princeton, New Jersey.

Greenfield, M.D. & Karandinos, M.G. (1976) Fecundity and longevity of *Synanthedon pictipes* under constant and fluctuating temperatures. *Environmental Entomology*, **5**, 883–887.

Luckinbill, L.S., Arking, R., Clare, M.J., Cirocco, W.C. & Buck, S.A. (1984) Selection for delayed senescence in *Drosophila melanogaster. Evolution*, **38**, 996–1003.

MacArthur, R.H. & Wilson, E.O. (1967) *The Theory of Island Biogeography.* Princeton University Press, Princeton, New Jersey.

McEvoy, P., Cox, C. & Coombs, E. (1991) Successful biological control of ragwort, *Senecio jacobaea*, by introduced insects in Oregon. *Ecological Applications*, **1**, 430–442.

Minkenberg, O.P.J.M. & Helderman, C.A.J. (1990) Effects of temperature on the life history of *Liriomyza bryoniae* (Diptera: Agromyzidae) on tomato. *Journal of Economic Entomology*, **83**, 117–125.

Myers, J.H. (1987) Population outbreaks of introduced insects: lessons from the biological control of weeds. *Insect Outbreaks* (ed. by P. Barbosa and J.C. Schultz), pp. 173–193. Academic Press, San Diego, California.

Nakamura, K. & Ohgushi, T. (1979) Studies on the population dynamics of a thistle-feeding lady beetle, *Henosepilachna pustulosa* (Kono) in a cool temperate climax forest. I. The estimation of adult population parameters by the marking, release and recapture method. *Researches on Population Ecology*, **20**, 297–314.

Nakamura, K. & Ohgushi, T. (1981) Studies on the population dynamics of a thistle-feeding lady beetle, *Henosepilachna pustulosa* (Kono) in a cool temperate climax forest. II. Life tables, key-factor analysis, and detection of regulatory mechanisms. *Researches on Population Ecology*, **23**, 210–231.

Nakamura, K. & Ohgushi, T. (1983) Studies on the population dynamics of a thistle-feeding lady beetle, *Henosepilachna pustulosa* (Kono) in a cool temperate climax forest. III. The spatial dynamics and the analysis of dispersal behaviour. *Researches on Population Ecology*, **25**, 1–19.

Ohgushi, T. (1983) *Population processes and life history strategy of an herbivorous lady beetle* Epilachna niponica. Dissertation. Kyoto University, Kyoto, Japan.

Ohgushi, T. (1986) Population dynamics of an herbivorous lady beetle, *Henosepilachna niponica*, in a seasonal environment. *Journal of Animal Ecology*, **55**, 861–879.

Ohgushi, T. (1991) Lifetime fitness and evolution of reproductive pattern in the herbivorous lady beetle. *Ecology*, **72**, 2110–2122.

Ohgushi, T. (1996) Consequences of adult size for survival and reproductive performance in a herbivorous ladybird beetle. *Ecological Entomology*, **21**, 47–55.

Ohgushi, T. & Sawada, H. (1995) Demographic attributes of an introduced herbivorous lady beetle. *Researches on Population Ecology*, **37**, 29–36.

Reznick, D.N. & Bryga, H. (1987) Life-history evolution in guppies (*Poecilia reticulata*): 1. Phenotypic and genetic changes in an introduction experiment. *Evolution*, **41**, 1370–1385.

Reznick, D.N., Bryga, H. & Endler, J.A. (1990) Experimentally induced life-history evolution in a natural population. *Nature*, **346**, 357–359.

Rose, M.R. (1984) Laboratory evolution of postponed senescence in *Drosophila melanogaster. Evolution*, **38**, 1004–1010.

Rose, M.R. (1991) *Evolutionary Biology of Aging.* Oxford University Press, New York.

Rose, M.R. & Charlesworth, B. (1981) Genetics of life history in *Drosophila melanogaster*. II. Exploratory selection experiments. *Genetics*, **97**, 187–196.

Sawada, H. (1984) *Population dynamics of an introduced herbivorous lady beetle*, Epilachna niponica. Dissertation. Kyoto University, Kyoto, Japan.

Sawada, H. & Ohgushi, T. (1994) An introduction experiment of an herbivorous lady beetle: characteristics of the adult population. *Researches on Population Ecology*, **36**, 29–36.

Stearns, S.C. (1983) A natural experiment in life-history evolution: field data on the introduction of mosquitofish (*Gambusia affinis*) to Hawaii. *Evolution*, **37**, 601–617.