Effects of artificial selection for reduced flight ability on survival rate and fecundity of *Harmonia axyridis* (Pallas) (Coleoptera: Coccinellidae)

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Abstract

The effects of artificial selection for reduced flight ability on the survival rate and fecundity of the ladybird beetle *Harmonia axyridis* were assessed. The hatching rate, emergence rate and fecundity of the flightless strain were significantly lower than the control in the 36th generation after artificial selection was started under laboratory conditions. In greenhouses containing cultivated eggplant in which the flightless strain was released, the incidence of fourth instar larvae, i.e., offspring of released adults, was delayed for 7 days compared with the control and commercial strain. These results suggest that artificial selection for reduced flight ability reduces the survival and reproductive characteristics of *H. axyridis*.

Key words: Inbreeding depression; biological control; *Harmonia axyridis*; quality control; flightless strain

INTRODUCTION

Augmentative biological control of ladybirds has been hampered by the tendency of adults to disperse because they are good flyers (Obata, 1986; Hodek et al., 1993; Tourniaire et al., 1999; Dixon, 2000). Marples et al. (1993) suggested that if ladybirds were poor flyers they would remain on a crop for longer and have potential in the long-term control of aphids. Flightless strains have been established from laboratory and field populations of the Coccinellids *Harmonia axyridis* (Pallas) (Ferran et al., 1998; Tourniaire et al., 2000; Seko et al., 2008), *Adalia bipunctata* (with a wingless morph) (L.) (Marples et al., 1993; Ueno et al., 2004; Lommen et al., 2005), and *Rhyzobius litura* (F.) (with a short-winged morph) (Hammond, 1985). Some of these strains are effective for augmentative biological control of aphids (Ferran et al., 1998; Weissenberger et al., 1999; Lommen et al., 2008; Seko et al., 2008).

Genetic processes, i.e., the founder effect, inbreeding, bottleneck effect, genetic drift, and unintentional selection for improved laboratory adaptation, have been a continuing concern in biological control and mass-rearing projects (Hopper et al., 1993; Roush and Hopper, 1995; Miyatake, 1998). Inbreeding increases the frequency of homozygotes and may result in decreased survival and reproductive characteristics by exposing deleterious recessive alleles (Hoy, 1985; Hopper et al., 1993; Falconer and Mackay, 1996; Roff, 1997; Lynch and Walsh, 1998; Frankham et al., 2002). Artificial selection for reduced flight ability, i.e., directional selection, tends to erode genetic variation (Falconer and Mackay, 1996; Roff, 1997) and loss of genetic diversity is related directly to the level of inbreeding (Frankham et al., 2002). Flightlessness is controlled by a homozygous recessive allele in some species of ladybirds (Marples et al., 1993; Ferran et al., 1998; Tourniaire et al., 2000). These factors can lead to reduced survival and reproductive characteristics of flightless ladybirds by inbreeding depression. The survival rate and fecundity of *H. axyridis* did not differ between adults of a flightless strain and those capable of flight (control strain) in the 15th generation after starting artificial selection (Tourniaire et al., 2000). However, with some biological control agents, depressed survival and reproductive characteristics have been observed in

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strains that have been colonized for a long time but not in recently colonized strains (Geden et al., 1992; De Clercq et al., 1998).

This study investigated the effects of long-term artificial selection for reduced flight ability on the survival and fecundity of *H. axyridis*. First, we evaluated whether differences in these characteristics occurred because of strain effects (flightless and control), generation effects (20th and 36th generations, i.e., ca 3 and 5 years, respectively, from starting artificial selection) or their interaction. The effects of artificial selection on reduced flight ability in *H. axyridis* were observed to compare changes in survival and reproductive characteristics over generations between the selected and non-selected strains, i.e., interactions between strain and generation.

Secondly, we researched the effectiveness of the flightless strain, the control strain, and ‘Nami-Top’ (a commercial *H. axyridis* strain) in controlling the cotton aphid, *Aphis gossypii* Glover, by releasing them in greenhouses.

**MATERIALS AND METHODS**

**Measurement of performance in *H. axyridis***. Artificial selection of a laboratory population of *H. axyridis* for reduced flight ability was conducted at the National Agricultural Research Center for Western Region of Japan from 2003 to 2007 and resulted in a flightless strain. Flight distance was measured in 7-day-old adults for 1 h as an indicator of flight ability using the flight mill system (Seko et al., 2008). Adults that recorded shorter flight distances (bottom 30% of 48 adults in each sex) were selected (Seko et al., 2008). Flightless adults have normal elytra and wings, but the rotor of the flight mill system did not spin because they could not flap their wings. A control strain was maintained using randomly selected offspring, thereby imposing no artificial selection on flight ability. These strains were established from 80 adult *H. axyridis* collected in Fukuyama (Western Japan, Lat. 34°28’S, Long. 133°23’E) during late April 2003. Both the flightless and control strains had been maintained using only 30–40 adults in each generation (Seko et al., 2008).

Several fitness parameters were assessed to evaluate the effects of artificial selection on the survival rate and fecundity of *H. axyridis*. In the 20th generation, 29 and 37 egg clusters on tissue paper were obtained from flightless and control strains of *H. axyridis*, respectively, which were kept at a density of 32 adults per plastic square container (16×22×8 cm). Each egg cluster was placed on a piece of wet filter paper in a Petri dish (9 cm in diameter) until the eggs hatched. The number of eggs in egg clusters was noted before hatching. The number of hatchlings was noted every 12 h for two days after the first hatching was confirmed. Hatchlings were removed immediately from Petri dishes to avoid cannibalism. A random sample of 100 hatchlings of each strain was individually introduced into a plastic cup (4.5 cm diameter and 3.5 cm high) and fed cucumber leaves and *A. gossypii* (50–70 individuals at the first-third instar stage and 100–150 individuals at fourth instar and adult stages). A piece of wet filter paper was placed at the bottom of the cup. Food was replenished at one-day intervals. Emergence rate was measured during individual rearing from hatching to emergence. Pupae were weighed using a Sartorius microbalance the day after pupation. Twenty-four and 22 pairs of one male and one female were randomly established the day after emergence for flightless and control strains, respectively. Fecundity was measured daily during the first 10 days after oviposition had been confirmed for each female. About 75% of total fecundity occurred during that time in the flightless strain of *H. axyridis* (Seko and Miura, unpublished). The same experiment was conducted in the 36th generation, in which the developmental period of immature stages and the preoviposition period of females were also monitored on a daily basis to facilitate the interpretation of the results of the release experiment in greenhouses. The total developmental period was defined as the period from hatching to adult emergence. The fitness parameters of the control strain were measured as well as those of the flightless strain. Sixteen and 24 mating pairs were obtained for flightless and control strains, respectively. These experiments were conducted under an LD of 16:8 at 25°C and 40–60% RH.

**Release experiment.** Four plots, i.e., the flightless strain, the control strain, ‘Nami-Top’ (a commercial strain of *H. axyridis*) and no treatment were used to evaluate whether artificial selection of *H. axyridis* for reduced flight ability affects the ef-
fectiveness of the control of \textit{A. gossypii} in small-scale greenhouses containing cultivated eggplants. The flight ability of ‘Nami-Top’ adults was suppressed by an artificial method developed by a Japanese company, Agrisect Inc. (Tezuka, 2003). The strain of ‘Nami-Top’ was maintained over 150 generations and the wings of adults were folded physically at emergence (Tezuka, personal communication).

The release experiment in greenhouses containing cultivated eggplant was carried out at the National Agricultural Research Center for Western Region of Japan in May 2007. Eggplant seedlings (cv. Chikuyô) with five leaves were transplanted into four plots on 9 May 2007. Each plot (about 4.0 m × 9.0 m for all treatments) consisted of two rows of plants with an 80 cm space between rows and 60 cm spaces between plants. Each plot contained 20 plants. \textit{A. gossypii} was confirmed for all treatments within one week of transplantation. Seven-day-old adults of 31st generation flightless and control strains and ‘Nami-Top’ adults (10 males and 10 females, respectively) were released onto eggplant stocks which observed the incidence of \textit{A. gossypii} on 21 and 28 May and 4, 11, and 18 June 2007. The numbers of \textit{A. gossypii} and \textit{H. axyridis} on eggplant were counted every three days. Six leaves were randomly selected per eggplant to estimate the numbers of \textit{A. gossypii}.

### Statistical analysis

Statistical analyses were performed using JMP, version 6.0.3 (SAS Institute, 2005). Normally distributed variables for the hatching rate (after arcsine transformation), pupa weight and fecundity were compared between strains in each generation by Student’s \(t\)-test and non-normally distributed variables for the developmental and preoviposition periods were compared by the Mann-Whitney \(U\)-test. The normality of the data was assessed using the Shapiro-Wilks test. Because the emergence rate is categorical data, the Chi-square test was performed. Interactions between strain and generation were examined by logistic regression analysis for the emergence rate and by two-way ANOVA for other traits.

Two-way ANOVA was analyzed to compare the incidence of \textit{A. gossypii} among greenhouses with dates as replicates, treatment as a fixed effect, stock nested in treatment, leaf nested in treatment, and stock as a random effect. Stock is the primary sampling unit and leaf is the secondary sampling unit. The numbers of \textit{H. axyridis} adults and fourth instar larvae per stock were compared by the same procedure with dates as replicates, treatment as a fixed effect, and stock nested in treatment as a random effect.

### RESULTS

#### Measurement of performance in \textit{H. axyridis}

Table 1 shows differences in viability, body size and fecundity between flightless and control \textit{H. axyridis} strains for each generation. The hatching rate of the flightless strain was significantly lower than that of the control strain in both generations (Table 1, 20th generation: \(df=64, t=3.11, p<0.01\); 36th generation: \(df=76, t=7.92, p<0.01\), Student’s \(t\)-test). The emergence rate did not differ between these strains in the 20th generation (Table 1, 20th generation: \(df=64, t=0.01, p>0.01\); 36th generation: \(df=76, t=0.01, p>0.01\), Student’s \(t\)-test).

![Table 1](attachment:image.png)

**Table 1. Differences in hatching rate, emergence rate, pupa weight and fecundity of \textit{H. axyridis} between the flightless strain and control in 20th and 36th generations**

\(a\) Number of egg clusters.

\(b\) Student’s \(t\)-test was conducted after arcsine transformation; **, \(p<0.01\); ***, \(p<0.001\).

\(c\) \(\chi^2\) test; ***, \(p<0.01\); ns, not significant.

\(d\) Student’s \(t\)-test; ***, \(p<0.001\); ns, not significant.

\(e\) Fecundity was measured over the first 10 days after starting oviposition.
In the 36th generation, it was significantly lower in the flightless strain (Table 1, $\chi^2=9.5$, $p<0.01$, Chi-square test). The pupa weight of males did not differ between strains in the 20th generation, but differed significantly in the 36th generation (Table 1, df=106, $t=0.55$, $p>0.05$; 36th generation: df=70, $t=4.80$, $p<0.001$, Student’s $t$-test). The pupa weight of females did not differ between strains (Table 1, 20th generation: df=106, $t=1.13$, $p<0.05$; 36th generation: df=47, $t=1.08$, $p<0.05$, Student’s $t$-test). No differences in fecundity were observed between these strains in the 20th generation (Table 1, df=44, $t=0.32$, $p>0.05$, Student’s $t$-test); however, the fecundity of the flightless strain was significantly lower than that of the control strain in the 36th generation (Table 1, df=38, $t=3.58$, $p<0.001$, Student’s $t$-test). The developmental period of the flightless strain was longer than that of the control strain (Table 2, male: $U=433.5$, $p<0.01$; female: $U=127.0$, $p<0.01$, Mann-Whitney $U$-test). The preoviposition period of the flightless strain was also longer than that of the control strain (Table 2, $U=102.5$, $p<0.01$, Mann-Whitney $U$-test). Two-way ANOVAs were significant in terms of the strain, generation, and interaction in the hatching rate, pupa weight of males, and fecundity (Table 3). For the pupa weight of females, two-way ANOVA was significant only in terms of strain (Table 3). Logistic regression analysis was significant in terms of strain in the emergence rate ($df=1$, $\chi^2=7.8$, $p<0.01$) and generation ($df=1$, $\chi^2=38.0$, $p<0.001$), but not the interaction ($df=1$, $\chi^2=0.34$, $p>0.05$).

### Table 2. Differences of developmental and preoviposition periods of H. axyridis between the flightless strain and control in the 36th generation

<table>
<thead>
<tr>
<th>Strain</th>
<th>Developmental period (day)$^{a,b,c}$</th>
<th>Preoviposition period (day)$^{a,c}$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male</td>
<td>Female</td>
</tr>
<tr>
<td>Flightless</td>
<td>16.0 (11.0–19.0) **</td>
<td>15.0 (14.0–20.0) **</td>
</tr>
<tr>
<td>Control</td>
<td>15.0 (13.0–19.0) **</td>
<td>14.5 (13.0–16.0) **</td>
</tr>
</tbody>
</table>

$^a$ Mann-Whitney $U$-test; $^{**}$, $p<0.01$.

$^b$ Developmental period is defined as days from hatching to emergence.

$^c$ Data are presented as the median (min.–max.).

### Table 3. Two-way ANOVAs for characteristics with viability and reproduction in H. axyridis

<table>
<thead>
<tr>
<th>Character</th>
<th>Factor</th>
<th>df</th>
<th>MS</th>
<th>$F$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hatching rate</td>
<td>Strain (S)</td>
<td>1</td>
<td>28,613.19</td>
<td>60.39***</td>
</tr>
<tr>
<td></td>
<td>Generation (G)</td>
<td>1</td>
<td>5,291.39</td>
<td>11.17**</td>
</tr>
<tr>
<td></td>
<td>S×G</td>
<td>1</td>
<td>6,414.28</td>
<td>13.54***</td>
</tr>
<tr>
<td></td>
<td>Error</td>
<td>154</td>
<td>473.80</td>
<td>—</td>
</tr>
<tr>
<td>Pupa weight (Male)</td>
<td>Strain (S)</td>
<td>1</td>
<td>161.44</td>
<td>7.98**</td>
</tr>
<tr>
<td></td>
<td>Generation (G)</td>
<td>1</td>
<td>426.70</td>
<td>21.09***</td>
</tr>
<tr>
<td></td>
<td>S×G</td>
<td>1</td>
<td>161.44</td>
<td>7.98**</td>
</tr>
<tr>
<td></td>
<td>Error</td>
<td>164</td>
<td>20.23</td>
<td>—</td>
</tr>
<tr>
<td>Pupa weight (Female)</td>
<td>Strain (S)</td>
<td>1</td>
<td>361.07</td>
<td>16.94***</td>
</tr>
<tr>
<td></td>
<td>Generation (G)</td>
<td>1</td>
<td>0.47</td>
<td>0.02 ns</td>
</tr>
<tr>
<td></td>
<td>S×G</td>
<td>1</td>
<td>45.15</td>
<td>2.12 ns</td>
</tr>
<tr>
<td></td>
<td>Error</td>
<td>153</td>
<td>21.32</td>
<td>—</td>
</tr>
<tr>
<td>Fecundity</td>
<td>Strain (S)</td>
<td>1</td>
<td>49,574.48</td>
<td>7.71**</td>
</tr>
<tr>
<td></td>
<td>Generation (G)</td>
<td>1</td>
<td>115,486.04</td>
<td>17.96***</td>
</tr>
<tr>
<td></td>
<td>S×G</td>
<td>1</td>
<td>35,217.81</td>
<td>5.48*</td>
</tr>
<tr>
<td></td>
<td>Error</td>
<td>153</td>
<td>5.48</td>
<td>—</td>
</tr>
</tbody>
</table>

*, $p<0.05$; **, $p<0.01$; ***, $p<0.001$; ns, not significant.
Release experiment

Figure 1 shows the numbers of (a) *A. gossypii*, (b) adult *H. axyridis*, and (c) fourth instar *H. axyridis* larvae. For the number of *A. gossypii*, two-way ANOVA was significant in all terms (treatment: $df=3$, $F=83.0$, $p<0.0001$; stock: $df=76$, $F=4.4$, $p<0.0001$; leaf: $df=400$, $F=2.2$, $p<0.0001$; dates: $df=18$, $F=379.1$, $p<0.0001$; dates $\times$ treatment: $df=54$, $F=64.3$, $p<0.0001$). The number of *A. gossypii* reached about 700 individuals per leaf in mid-June in the no-treatment plot. Numbers of *A. gossypii* were suppressed in treatments in which adult *H. axyridis* were released. The ‘Nami-Top’ was most effective in controlling *A. gossypii*. In the treatment in which the flightless strain was released, the incidence of fourth instar larvae, i.e., the offspring of released adults, was delayed for 7 days compared with other treatments. For the numbers of adult *H. axyridis*, two-way ANOVA was significant in terms of stock ($df=57$, $F=1.5$, $p<0.05$), dates ($df=15$, $F=11.3$, $p<0.0001$) and dates $\times$ treatment ($df=30$, $F=3.5$, $p<0.0001$), but not in term of treatment ($df=2$, $F=0.4$, $p>0.05$). For the numbers of fourth instar larvae *H. axyridis*, it was significant in terms of dates ($df=30$, $F=2.1$, $p<0.001$) and dates $\times$ treatment ($df=30$, $F=2.1$, $p<0.001$), but not in terms of stock ($df=57$, $F=0.8$, $p>0.05$). It was marginally significant in terms of treatment ($df=2$, $F=3.1$, $p=0.054$).

DISCUSSION

Inbreeding accumulates over time and more rapidly in smaller populations (Frankham et al., 2002). The characteristics most closely related to reproductive fitness show greater inbreeding depression than those that are peripherally related to fitness (Mousseau and Roff, 1987; Falconer and Mackay, 1996; Lynch and Walsh, 1998). The effects of inbreeding have been described as potential deterrents to genetic improvement projects (Hoy, 1985). If the flightlessness of ladybirds is controlled by recessive alleles, artificial selection for reduced flight ability should increase the level of homozygosity and promote exposure to deleterious recessive alleles. In our study, no difference was observed between flightless and control strains in the emergence rate and fecundity in the 20th generation. The emergence and fecundity rates were similar to the initial rates for the control strain (emergence rate: 0.84, fecundity: 215.9 $\pm$ 97.2 in the 3rd generation, Seko and Miura, unpublished). On the other hand, these parameters differed between strains in the 36th generation. Interactions between strain and generation were detected for the hatching rate, pupa weight of males, and fecundity. These results suggest that artificial selection for reduced flight ability exerts deleterious inbreeding effects on the survival and reproductive characteristics of *H. axyridis*. The frequency of flightless adults was
about 0.7 in the 20th generation, but was 1.0 in the 30th generation (Seko et al., 2008). Because genetic variation in fitness parameters persisted through 20 generations, exposure to deleterious recessive alleles might have been less in the 20th generation than the 36th generation.

Selection for a trait can affect other traits via genetic correlation caused by pleiotropic effects or linkage (Stearns, 1992; Arnold, 1994; Falconer and Mackay, 1996; Roff, 1997). A correlated response would also be expected as a result of artificial selection for reduced flight ability in *H. axyridis*. A genetic trade-off between egg production and wing formation has been demonstrated in some species (Roff, 1990; Dingle, 1996; Roff and Bradford, 1996); however, the fecundity of the flightless strain was lower than that of the control strain in our study. In wingless adults of *A. bipunctata*, total fecundity was lower and longevity after emergence was shorter than that of winged adults (Ueno et al., 2004). A trade-off between flight and reproductive characteristics may be masked by the deleterious effects of inbreeding in these ladybirds.

In our release experiment, the commercial ‘Nami-Top’ strain was most effective in suppressing the incidence of *A. gossypii*. In the treatment in which the flightless strain was released, the incidence of fourth instar larvae was delayed for 7 days compared with other treatments. In our study, extension of the developmental and preoviposition periods and a decrease in the hatching rate, emergence rate and fecundity were observed for the flightless strain. The results of the release experiment showed that depression of these fitness parameters can affect the effectiveness of aphid control. Another possible explanation for the observed results is that artificial selection for reduced flight ability may affect the function involved with host searching in *H. axyridis*. Prey search and feeding durations for pea aphids (*Acrithosiphon pisum*) tend to be longer in a flightless strain of *H. axyridis* than in a control strain (Tourniaire et al., 1999).

Other mechanisms that may cause changes in the characteristics related to fitness are the founder effect, bottleneck effect, genetic drift, and unintentional selection for improved laboratory adaptation (Hopper et al., 1993; Roush and Hopper, 1995; Miyatake, 1998; Frankham et al., 2002). In our study, the founder effect could be ignored as the initial population was derived from 80 adults collected in the field (Seko et al., 2008). Roush and Hopper (1995) suggested that 46 family lines are needed to preserve alleles that seem most likely to be important in field performance for diploid species. The bottleneck effect may have been involved because the flightless strain was derived from an isogenic line that was established in the seventh generation to promote homozygosis (Seko et al., 2008). In a Palaeartic ladybeetle, *Propylea quatuordecimpunctata*, the fitness parameters of inbred lines decreased after only one generation of sibling mating (Morjan et al., 1999). However, none of the measured fitness parameters, except the hatching rate, differed in our study after 20 generations of selection. Hopper et al. (1993) stated that severe bottlenecks do not necessarily decrease additive genetic variance in quantitative traits likely to affect fitness. Loss of genetic variation arises predominantly from sustained reductions in population size, rather than single-generation bottlenecks (Frankham et al., 2002). The effects of artificial selection for reduced flight ability may have been greater than those of a bottleneck during the establishment of the isogenic line of *H. axyridis*. Unintentional selection for improved laboratory adaptation is unlikely to have affected the characteristics related to fitness in our study because there were no negative genetic correlations between the same traits across different resource environments in *H. axyridis* (Ueno, 2003). On the other hand, genetic drift may have occurred because of the small population size (Hopper et al., 1993; Falconer and Mackay, 1996; Frankham et al., 2002). Both the flightless and control strains had been maintained using only 30–40 adults in each generation (Seko et al., 2008). A decrease in the emergence rate of the control strain may have caused genetic drift in our study; however, we could not determine the possible effects of genetic drift because there were no replicates of these strains. Other lines should be established to facilitate research on the effects of genetic drift in a small population of *H. axyridis*.

We conclude that artificial selection for reduced flight ability may depress survival and reproductive characteristics in *H. axyridis*. The results of release experiment suggest that depression of these fitness parameters affects the effectiveness of aphid control. Flightless adults of *H. axyridis* were effective in open fields containing cultivated eggplants (Seko et al., 2008); however, the number of *A.
**Fitness Parameters of a Flightless Lady Beetle**


Mousseau, T. A. and D. A. Roff (1987) Natural-selection and...


