A natural flightless mutation in the ladybird, *Harmonia axyridis*

R. Tourniaire, A. Ferran*, L. Giuge, C. Piotte & J. Gambier
INRA, Laboratory of Invertebrate Biology, 37 boulevard du Cap, 06600 Antibes, France
*Author for correspondence

Abstract

A homozygous flightless strain, obtained by selection from a laboratory population of *Harmonia axyridis* Pallas (Coleoptera, Coccinellidae), has been reared under controlled conditions since 1982 after sampling in China. The adults of this flightless strain have normal elytra and wings but drop almost vertically when they attempt to fly. The origin of this mutation, whether laboratory or natural, is discussed. As the mutation does not affect the fitness of the beetles, this flightless strain can be used in biological control. Releasing flightless adults rather than larvae should provide the potential for more continuous control of aphids by both larvae and adults.

Introduction

Introduced entomophagous insects are rarely perfectly adapted for use in biological control. Four decades ago, the genetic improvement of entomophagous insects, via artificial selection, was proposed as a method of enhancing their effectiveness as biological control agents (DeBach, 1958). Agents have been selected for tolerance of low or high temperatures (Wilkes, 1947; White et al., 1970), mass rearing on a new prey (Allen, 1954), non diapause (Field & Hoy, 1986) or resistance to pesticides (Fournier et al., 1988; Grafton-Cardwell & Hoy, 1986; Hoy & Cave, 1989; Presnail & Hoy, 1992).

Many other beneficial ecological or behavioural traits could be selected. Shortening the developmental time, enhancing progeny production, modifying the sex ratio in favour of females, or altering habitat preference could improve the efficiency of entomophagous insects (Hoy, 1990). There is a lack of understanding of the attributes of entomophagous insects which make some of them ineffective as biological control agents. For this reason, genetic improvement projects have generally been restricted to two traits: pesticide resistance and non diapause.

In aphidophagous ladybirds, the critical trait is their ability to fly (Hodek et al., 1993). When used in biological control programmes they frequently leave outdoor and greenhouse crops, so that regular reintroductions are necessary (Marples et al., 1993). Because the adults fly, larvae are usually used to control aphid populations, but their effectiveness is constrained by their short developmental time (Ferran et al., 1996).

If adults were poor flyers they would remain in a crop for longer and provide the potential for more long term control (Marples et al., 1993). To produce flightless individuals of *Harmonia axyridis* Pallas, adults were initially fed a mutagenic substance and then selected in subsequent generations in one of two ways: (1) Selection of those with malformed elytra and wings (Ferran et al., 1998) or (2) Selection of those with normal elytra and wings but unable to fly (Tourniaire et al., 2000).

Flightless adults also occur in natural populations. For instance, a male of *Adalia bipunctata* L. with an inherited malformation of its elytra and wings has been recorded. However, the scarcity of such mutations and their low fitness prevent their use in biological control programmes (Marples et al., 1993). Here a flightless line with normal elytra and wings have been selected from the control laboratory population without use of mutagenic agents. This paper describes the selection procedure and the take-off behaviour of this new flightless strain, and compares its biologi-

cal and reproductive abilities with those of the control population of *H. axyridis*.

Material and methods

Selection of flightless individuals. The aphidophagous ladybird, *H. axyridis*, was introduced into France from China in 1982 (approximately 500 founders) and has been reared in mass laboratory cultures on a substitute prey, the eggs of *Ephestia kuehniella* Zeller (Lepidoptera, Pyralidae).

Three groups of 200 six day old adults (as many males as females) were randomly drawn from the control stock and placed in a device under climatic conditions that are optimal for flight (patent no. 96 14859). The device allowes the separation of flightless adults with normal elytra and wings which remain in the device, from those that can fly out of the device. These flightless adults were mated and their adult progeny similarly selected for flightlessness for a number of generations. After fifteen generations of selection the entire stock was flightless, no adults flying out of the device. Some beetles of the fourteenth generation were reared without further selection for three generations to test their homozygosity.

The results were expressed as the mean proportion of adults that did not fly. A Chi-squared test was used to compare the number of flightless to flying insects in the two populations.

Take-off behaviour. The take-off behaviour of the flightless and control population was compared outdoors on warm sunny days (end of June and July). A 5 m × 5 m area divided into 100 squares (0.5 m × 0.5 m), was delineated by means of strings placed on the ground. A white painted wooden stick (height: 0.6 m, diameter: 0.3 cm) was placed vertically in the middle of this area. Most of the adults released at the bottom of the stick climbed to the top and then jumped off. The squares in which one hundred adults of each population either landed or flew over when leaving the area were recorded. The results were expressed as the number landing in each square or flying over when leaving the area.

Biological performances of the selected population. The biological characteristics of 90 larvae of the selected population (generation 15), reared from the egg stage were compared with those of the same number of control larvae. The percentage mortality during larval

development, duration of development (days), adult weight (mg), and sex-ratio (proportion of females) were recorded.

Thirty pairs of selected and control adults were isolated and reared for 30 days under the same trophic conditions. The following biological parameters were recorded: percentage mortality, frequency of infertile pairs, number of days on which eggs, were laid, fecundity in terms of eggs and larvae and percentage egg hatch. This experiment was carried out at 21 °C, ± 1 °C, 70 to 80% r.h. and a 16-h day length. Results were expressed as averages plus the corresponding confidence interval (P<0.05). χ^2-squared and *t*-tests were used to compare averages and actual data (sex-ratio, mortality) respectively, in the two populations.

Results

Selection of flightless individuals. Initially in the control population (C), the frequency of adults that did not fly, was about 10% (Figure 1). With selection, the frequency of flightless adults quickly increased in the second and third generation, then fluctuated between 80% to 90% and reached 90% to 100% in the fourteenth generation. In the last three generations, there was no difference in the frequency of flightless adults in the selected and non selected populations ($\chi^2 = 0.58$; P = 0.90). It is thus likely that by this stage of the selection programme all the individuals were homozygous for flightlessness.

Take-off behaviour. Of the hundred control adults that left the stick tip, 19% landed near the base of the stick and 81% flew out of the area (Figure 2). All the flightless adults dropped vertically, landing close to the base of the stick. After take-off, these adults opened their elytra and extended their wings but did not fly.

Biological performance of the selected population. Most of the biological characteristics of the larvae (Table 1) and adults (Table 2) from the control and selected populations were very similar. The duration of development of the selected beetles was slightly shorter, by about half a day, and the hatch-rate of their eggs was approximately 10% lower than in the control population. Nevertheless, this lower hatch-rate did not prevent the mass rearing of the selected population.
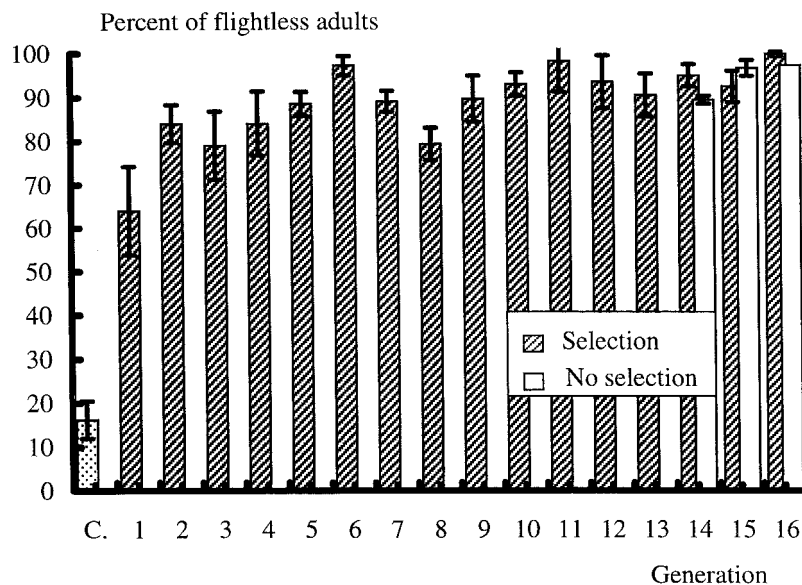
Percent of flightless adults

Figure 1. Percentage of flightless individuals of *H. axyridis* in a laboratory population (shaded columns: percentage in a selected population; open columns: percentage of flightless adults in a population in which from the thirteenth generation the progeny were not subjected to selection; C: control or laboratory population).

Discussion

A flightless strain with normal elytra and wings was selected from the laboratory population of *H. axyridis*. The flightless adults showed the same innate tendency to climb up a vertical stick (positive phototaxis and negative geotaxis) prior to flying as normal adults do. However, when they attempted to fly, they opened their elytra and wings but then fell almost vertically. This incapacity to fly is associated with specific structural modifications of dorsal longitudinal, dorsoventral and basal indirect wing muscles (Tourniaire, unpubl.). The Z-discs in the myofibrils are either deformed, fragmented or absent, and the myofibrils themselves are heterogeneous in size and shape.

The biological and reproductive abilities of the flightless *H. axyridis* population are very similar to those of the normal one. In some other insect species, flightless adults have a shorter preoviposition period and lay more eggs than normal adults (Roff, 1977; Harrison, 1980). In *H. axyridis* fligthless adults, only the hatching rate was slightly lower than in normal ones. It seems there is an association between flightlessness and the reproductive performance in this beetle. The hatching rate of adults with drastic morphological elytrae and wing malformations (17.4%) was lower than in adults with only structural modifications of the wing muscles (64.6%) (Ferran et al., 1998). This

relationship may be due to one gene influencing more than one characteristic (pleiotropy) or several genes linked on the same chromosome, which means that selection for one gene automatically induces selection for the associated gene or genes (Bartlett, 1984).

The mutation is recessive, affectes males and females, and only homozygotes show the 'flightless' trait (R. Tourniaire, unpubl.). The rapid response to selection suggests a single gene or perhaps two main genes controlling muscle development. However, the slow subsequent increase of flightless adult frequency could indicate the participation of a polygenic modifier system as well (M. Majerus, pers. commun.). Large numbers of adults are generally required to select for a single mutation as it was shown in *Drosophila melanogaster* (Cripps et al., 1994). The high level of fligthless adults found in the normal population of *H. axyridis* may result from inbreeding for many years (Joslyn, 1984). The easiness with which we were able to select for a flightless population may be due to the high initial frequency of flightlessness in the laboratory population. To test this, several wild populations of this ladybird from its area of origin should be checked for the incidence of flightlessness.

The main question remains the origin of this mutation: either it was present in the population (approximately 500 adults) introduced from China in 1982 or it resulted from the continuous rearing of this ladybird

Table 1. Biological characteristics of control and selected populations of *H. axyridis*

Biological parameters	Populations		Statistical tests
	Control	Selected	
Number of larvae	90	90	
Mortality (%)	8.9 ± 2.0	7.8 ± 3.7	$\chi^2 = 0.1$, P = 0.1
Duration of development (days)	17.5 ± 0.2	16.9 ± 0.2	$t = 4.5$, P = 0.001*
Adult weight (mg)			
females	36.7 ± 1.0	36.8 ± 1.0	F = 2.3, P = 0.12 (1)
males	32.2 ± 1.0	30.7 ± 0.7	
Sex-ratio	53	53	$\chi^2 = 0.2$, P = 0.07

*significant difference. (1): comparison between the two populations, whatever the sex. Females were significantly heavier than males (F = 134, P<0.0001).

Table 2. Biological and reproductive characteristics of control and selected populations of *H. axyridis*

Biologicals parameters	Populations		Statistical tests
	Control	Selected	
Number of adult pairs	30	30	
Mortality (% in 30 days)	0	3.3	
Ovarian growth (days)	11.7 ± 0.7	10.7 ± 0.6	$t = 1.1$, P = 0.34
Infertile pairs (%)	0	3.3	
Number of days on which eggs were laid (in 30 days)	19.5 ± 1.1	19.0 ± 1.4	$t = 0.3$, P = 0.77
Total number of eggs (per female, in 30 days)	853 ± 80	881 ± 83	$t = 1.7$, P = 0.18
Total number of larvae (per female, in 30 days)	630 ± 11	583 ± 81	$t = 1.6$, P = 0.21
Percentage hatch (%)	74.3 ± 6.7	64.6 ± 1.6	$t = 3.2$, P = 0.05

*Significant difference.

under standardized trophic and climatic conditions. 'Flightless' mutations were sometimes discovered in wild ladybird populations. For example, Marples et al. (1993) found a male of *A. bipunctata* L. with a wing malformation. The corresponding mutation was recessive and lethal. In the ladybird, *Rhyzobius litura* F. a genetic polymorphism with brachypterous and full-winged adults was described by Hammond (1985). Changes in the duration of development, fecundity, prey suitability and acceptance, locomotion, longevity, male sterility, sex ratio, and temperature preference and tolerance of laboratory reared biological control agents have been revealed (reviewed in Hopper et al., 1993). In some insects, the ability to fly was severely restricted by laboratory rearing conditions (Bartlett, 1984). Three processes may be involved in these changes, viz., drift, unintentional selection and inbreeding. In most cases, biological and physiological changes seem to involve temporary adaptations rather than mutations (Hopper et al., 1993).

Accepting that flightless individuals are present in wild populations, then some populations of *H. axyridis* will contain homozygous flightless beetles and heterozygous and homozygous adults able to fly. A population of *H. axyridis* homozygous for flight was recently produced by selection in the laboratory using isofemale lines (R. Tourniaire, unpubl.).

H. axyridis is a migratory species (Obata, 1986). In late autumn, heterozygous and flying homozygous individuals migrate from feeding to overwintering sites in the mountains, whereas the homozygous flightless adults remain in the crops. Most of the latter probably die due to lack of essential preys. Nevertheless, it could not be excluded that some of them may survive in summer and then hibernate in diapause till the next spring, this species being able to feed on alter-
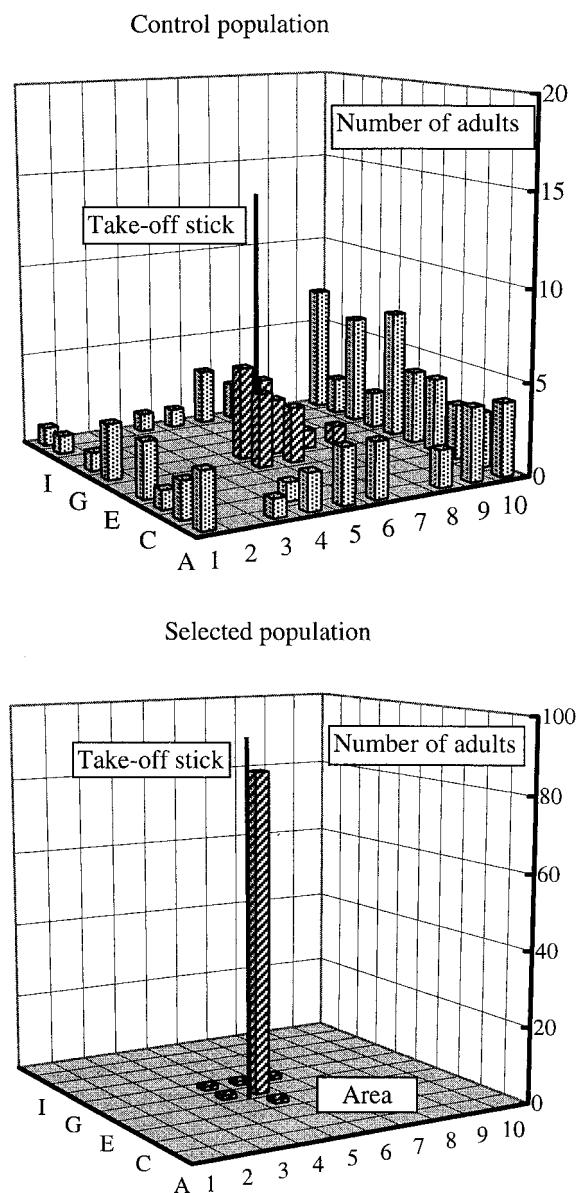
Control population

Selected population

Figure 2. Take-off behaviour of control and flightless adults of *H. axyridis* (dotted columns: number of adults that flew out of the release area; hatched columns: number of adults that landed in the release area).

able diapause sites, fungal diseases, and mortality due to temporarily extreme climatic conditions. Remaining in crops may be an alternative, probably safer, overwintering strategy for this species.

The flightless population can be mass reared for use in biological control programmes. As adults tend to fly away from the treated crops, only larvae of this species have so far been used in biological control programmes in Europe. Now, it is possible to reduce the costs of biological treatments with *H. axyridis* by releasing small numbers of flightless adults onto the crops where their progeny will control aphid populations over longer periods of time. Moreover, the predatory activity of the flightless adults will aid the larvae in controlling the aphid population.

Acknowledgements

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native preys and extrafloral nectar (Pemberton et al., 1993, Sakurai et al., 1992). As the frequency of flightless adults in wild populations at the end of a year is unknown, it is difficult to estimate whether the overwintering of flightless adults in crops contribute to the persistance of flightnessness in populations of this ladybird beetle. Migration to overwintering sites is risky for ladybirds because of predation, failure to find suit-

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