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An artificial non-flying mutation to improve the efficiency of the ladybird *Harmonia axyridis* in biological control of aphids

A. FERRAN[1], L. GIUGE[1], R. TOURNIAIRE[1], J. GAMBIER[1] and D. FOURNIER[2]
[1]*I.N.R.A., Laboratory of Invertebrate Biology, 37 boulevard du Cap, 06600 Antibes, France;*
[2]*Paul Sabatier University, Laboratory of Entomology, 118 route de Narbonne, 31062 Toulouse, France*

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Abstract. The use of coccinellids in the biological control of aphids is restricted to the release of larvae because adults tend to fly away. Non-flying adults may stay longer in one place and so they and their progeny could give longer term protection to plants. This work is an attempt to produce a non-flying population by the use of a chemical mutagen and selection of adults with wing malformations through their subsequent generations. These adults are characterized by open elytra and extended wings. Some general features of this mutation were disclosed. The mutation is either unexpressed or results in malformed wings. It also seems recessive and lethal when homozygous. The adults with the mutation suffered a high level of mortality and a drastic reduction in reproductive capacity that prevents their mass rearing for biological control. This study revealed a negative relationship between wing malformations and reproductive capacity. Nevertheless, when adults with the mutation were released in greenhouses containing cucumbers infested with the aphid *Aphis gossypii*, they remained on the plants in higher numbers and laid eggs over a longer period of time than the control adults but their progeny were less numerous.

Résumé. La lutte biologique avec la coccinelle *Harmonia axyridis* contre les pucerons fait uniquement appel aux larves puisque les adultes quittent généralement les cultures. Des adultes incapables de voler pourraient rester plus longtemps dans les cultures assurant une protection phytosanitaire de longue durée grâce leur descendance. Ce travail avait pour objectif de produire une population inapte au vol par mutagenèse chimique et sélection au cours des générations successives des adultes présentant des malformations alaires invalidantes. Les adultes obtenus sont caractérisés par des élytres ouverts et des ailes déployées en permanence. Quelques particularités de cette mutation ont été mises en évidence. La mutation obtenue peut ne pas s'exprimer ou, au contraire, se manifester sous forme de trois phénotypes différents. Cette mutation paraît être récessive, dépendante de la température et léthale à l'état homozygote. Les adultes mutés sont caractérisés par une forte mortalité et par une réduction drastique de leurs potentialités reproductrices. En conséquence, l'élevage en masse de la population mutée dans une perspective de lutte biologique ne peut être envisagée. Il existe manifestement une relation entre l'existence de malformations alaires et la diminution des capacités reproductrices chez les adultes. Cependant, quand les adultes mutés sont lâchés en serres, ils y restent en plus grand nombre, y pondent plus longtemps que ne le font des adultes témoins mais leur descendance est moins nombreuse.

Key words: abnormal wings, adult, biological control, Coccinellidae, Coleoptera, mutagenesis, mutation

Introduction

Aphidophagous ladybirds (Col., Coccinellidae) are good flyers. Adults undertake foraging flights within and between fields when aphids are present, migratory flights to summer aestivo-hibernating sites and in spring disperse to cultivated areas (Hodek, 1967; Iperti, 1986; Ferran et al., 1989; Hodek et al., 1993).

Aphidophagous ladybirds consume large quantities of aphids (Honek, 1980, 1982; Ferran et al., 1984; Schanderl et al., 1985). They have been used to control aphid populations in greenhouses and field crops (Deng et al., 1987; Ghanin and EL-Adl, 1991). Because the adults fly, mainly egg-clusters or larvae are used and 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 a more long term control (Marples et al., 1993).

Adults with wing malformations are sometimes observed in natural insect populations (Hammond, 1985). If they are of teratological origin they are not transmitted to their progeny but most are likely to be a consequence of a mutation and inherited (Majerus and Kearns, 1989; Marples et al., 1993). The scarcity of these mutations and the low fitness of these adults prevents their mass production (Marples et al., 1993).

Wing malformations have been obtained by treating larvae with an insect growth regulator (Mordue and Blackwell, 1993) or by feeding a mutagenic substance to adults. In this study, a chemical mutagen was applied to the ladybeetle *H. axyridis* Pallas and in subsequent generations only the adults with wing malformations were used for breeding.

This work describes some of the features of the mutants (homozygosity, phenotypic plasticity, response to rearing temperature), and the performance of the non-flying adults. It also reports the results of an ecological experiment in which control and mutated ladybirds were released in two separated greenhouses containing cucumbers infested with the aphid *Aphis gossypii* Glover.

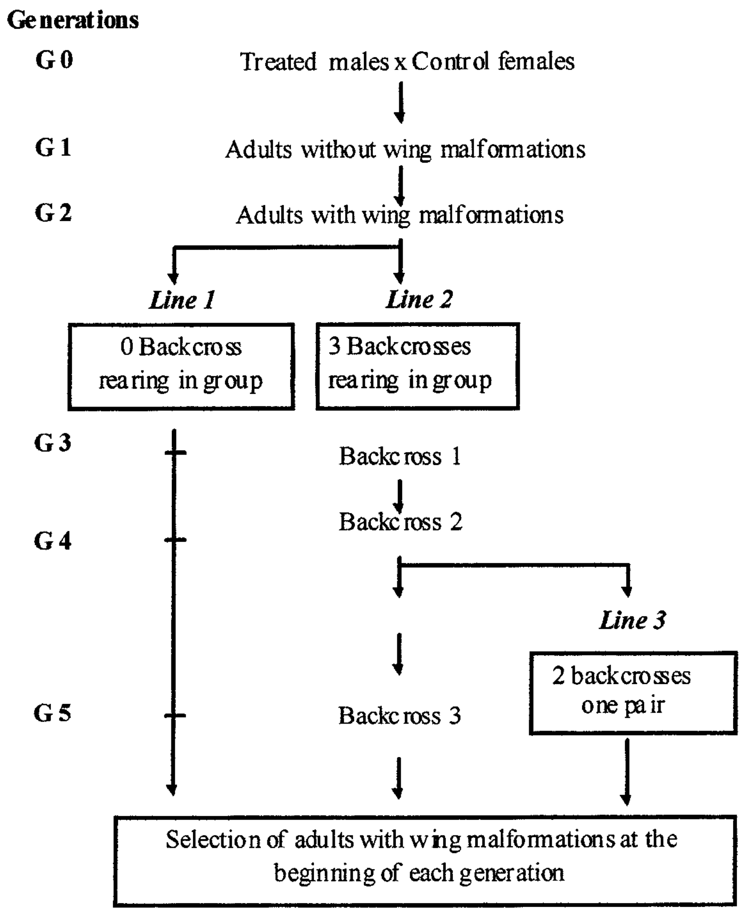

Figure 1. Method of selection of *Harmonia axyridis* adults with wing malformations after ingestion of a mutagen.

Material and methods

Method of mutagenesis and selection

The Asiatic polyphagous ladybird *Harmonia axyridis* was first reared in a French laboratory in 1982, after which it has been continuously reared (more than 200 generations) on the eggs of *Ephestia kuehniella* Zeller (Lep., Pyralidae) (Schanderl et al., 1985) and mass produced for use in biological control programmes against aphids (Ferran et al., 1996).

The chemical and the method used to obtain the wing malformation has been patented (No. 9614859). Only young males were treated with the mutagen and mated with virgin females in a plastic cage and their offspring reared at 20 °C. Wing malformations only appeared in the second generation (Figure 1). From the F2 generation, three lines were isolated. Line 1 was multiplied without backcrossing. At each generation, 30 pairs of virgin adults with wing malformations were selected and reared in a group to produce the next generation. Males of line 2 were crossed three times (F3 to F5) with wild females in order to eliminate potential lethal mutations and then selection was performed as in line 1. Line 3 was obtained from one adult pair of line 2 (F3) which was selected as in line 1, except that the fourth, ninth and tenth generations were initiated only from one pair instead of 30.

Characterization of the mutation

Adults were assigned to one of three phenotypes. One (P1) or both wings permanently protruded beyond the elytra and either parallel (P2) or more or less perpendicular (P3) to the saggittal axis of the body. Thirty adults of each phenotype (line 1, F13) were crossed and the frequency of the three wing phenotypes in a sample of 100 of their progeny estimated.

In the tenth generation line 2 was also tested for homozygosity. A sub-population was initiated using the same number (30) of abnormal and normal immature adults and the frequency of wing malformations in 100 adults of their progeny estimated.

The effect of temperature on the frequency of wing malformations was studied by rearing young first instar larvae of line 2 (F7) to maturity at four constant temperatures (15, 18, 23 and 28 °C). Three groups of 50 larvae were reared at each temperature.

Biological performance of the mutants

Biological characteristics of 30 pairs with wing malformations (line 2, F7) were compared with those of the same number of control adults. Mortality during sexual maturation and the first ten days of egg-laying was recorded. Time taken to mature their ovaries (in days) was estimated from the time taken by each pair to lay their first egg-cluster. Fecundity was expressed by the percentage of pairs which laid eggs and the mean number of eggs they laid in the first ten days of their egg-laying period. Egg hatch rate per pair was the percentage of live larvae obtained from these eggs.

Effectiveness of the mutants in biological control

This experiment was done in two greenhouses each containing two rows of 14 cucumbers growing in artificial soil and fed a nutrient solution. They were infested with 500 *A. gossypii* from a laboratory culture. Five young fertile

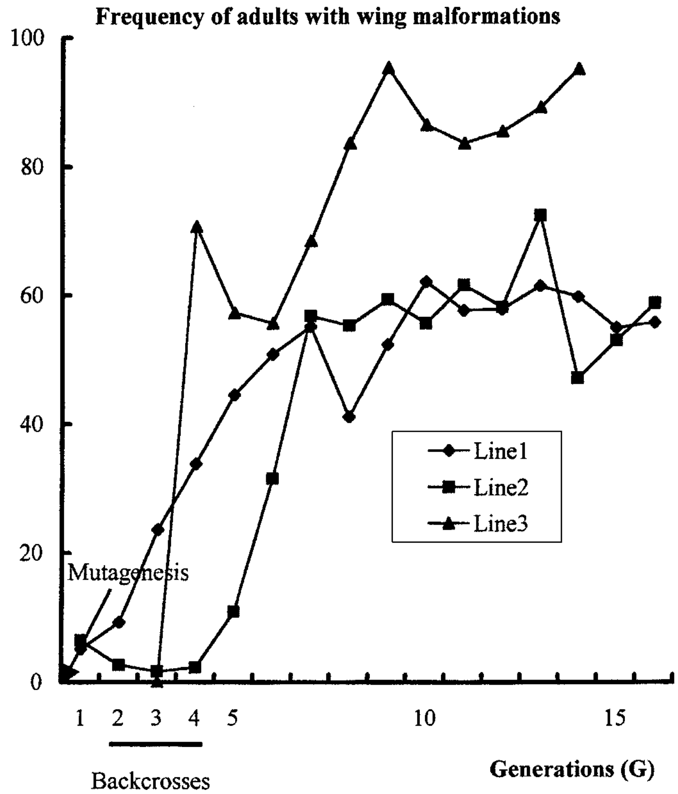

Figure 2. Variation of mutant adult frequency through generations (F) in the different lines (see Material and methods for line characteristics).

mutant (greenhouse 1) or control (greenhouse 2) *H. axyridis* females and males were released on each plant 10 days after they were infested with aphids.

Aphid number was visually estimated using a method based on that of Lapchin and Boll (1996). One day before the ladybeetles were released and then twice a week thereafter each leaf was categorized as either: class 0 (0 aphid), class 1 (1 aphid to several colonies), class 2 (ca. 25% of the leaf area infested), class 3 (ca. 50% of the leaf area infested), class 4 (ca. 75% of the leaf area infested), class 5 (small leaf uniformly infested – leaf length and width shorter than 20 cm), and class 6 (large leaf uniformly infested – leaf length and width longer than 20 cm). The aphid population was expressed in terms of the frequency of leaves in the different abundance classes on each plant and the mean frequency was calculated for all the plants in each greenhouse. The total number of coccinellids (egg-clusters, larvae, and adults) on

Table 1. Frequency of wing malformations in the progeny of adults that differed in their wing phenotype (parental population: 30 pairs per wing phenotype; frequency estimated from 100 adults per phenotype, see Material and methods for definition of phenotypes)

Parental phenotypes	Progeny phenotypes			Progeny phenotypes
	P1	P2	P3	
P1	6	12	40	42
P2	4	10	50	36
P3	4	16	48	32

each plant was recorded twice a week from the introduction of the adults to the pupation of their larvae.

The results were expressed as means with their corresponding standard-error ($p < 0.05$). Comparisons were done using different statistical tests: χ^2 test (frequency comparison), t test (comparison of two means) and F test (comparison of several means).

These experiments used control and mutant adults that were reared on the eggs of *Ephestia kuehniella* Zeller.

Results

Incidence of mutant adults

In line 1 (without backcrossing), the frequency of abnormal adults increased up to the eighth generation (F8: 50.9%) then varied between 50% and 60% (Figure 2). Line 2 (with three backcrosses) showed the same trend although the frequency increased more slowy than in line 1 probably due to the dilution that resulted from the backcrosses. In line 3, frequency was high from the fourth generation, which was initiated from one adult pair, then decreased probably because selection favored less abnormal phenotypes when stocks were reared in a group (F5 to F8). The frequency increased again in the F9 for the same reason and remained high in the following generations (83.6% to 95.2%). All the egg-clusters failed to hatch in the F14 and as a consequence line 3 died out.

Characterization of the mutants

The frequency of the three phenotypes observed in various lines was independent of the parental phenotype ($\chi^2 = 4.67$, df = 6, $p = 0.59$) (Table 1). A

Table 2. Effect of larval rearing temperatures on the frequency of adults with wing malformations (larvae: line 2, F7; control: mass rearing of larvae)

Population	Pre-imaginal rearing temperature (°C)			
	15	18	23	28
Mutated (%)	64.5 ± 3.9*	69.0 ± 8.7	63.5 ± 7.3	29.8 ± 6.1
Control (%)	3.0	4.5	0	0

*(± sd).

fourth phenotype, in which the elytrae and wings were crumpled and shorter than the abdomen, sometimes looking like stumps, was observed at the start of the experiment. This particular phenotype quickly disappeared probably because it was lethal. Such phenotypic diversity has been observed in the ladybeetle *Adalia bipunctata* L. (Marples et al., 1993).

Only 15.5% ± 7.6% of the progeny of the subpopulation used for homozygosity had wing malformations, compared with 55.5% in the parental population (Line 2, F10). This result suggested that line 2 and probably line 1 were heterogeneous in spite of the stability of the frequency after F8 (Figure 2).

In all lines, adults without wing malformations (40–50% per generation) were morphologically similar to control adults. When such adults (line 3, F11) were mated, 50.9% of their progeny had wing malformations. It can be concluded that most of these adults carried the mutation.

When larvae from the mutated population (line 2, F11) where reared at different temperatures, the mortality was not different (8–10%). The number of adults with wing malformations was constant between 15 and 23 °C and significantly less at 28 °C ($\chi^2 = 6.91$, df = 3, $p = 0.07$) (Table 2). Therefore, the expression of the mutation seems to depend on rearing temperature. Some wing malformations were also observed in the control population when larvae were reared at 15 and 18 °C.

Biological performance of the mutants

Compared to the controls, adults with abnormal wings suffered a greater mortality, particularly the females, and spent a longer time in the sexual maturation period (t = 2.98, df = 53, $p < 0.01$) (Table 3). Although mating was observed in all mutated single pairs, the frequency of egg-laying pairs (control: 96.6 ± 5.3; mutated adults: 36.0 ± 4.8) and the mean total number of eggs laid by fertile pairs was clearly reduced (t = 9.3; df = 33, $p < 0.001$). The most drastic consequence of the mutation on adult reproductive capacity

Table 3. Biological characteristics of adults with wing malformations (mutant adults from line 2, F7, control from mass rearing)

Biological parameters	Adults	
	Control	Mutated
Number of pairs	30	30
Mortality (%)		
females	3.3 ± 5.2*	30.0 ± 10.2
males	3.3 ± 2.2	5.3 ± 4.3
Ovarian growth (in days)	8.0 ± 1.9	10.8 ± 4.7
% of pairs laying eggs	96.6 ± 5.3	36.0 ± 4.8
Total fecundity (in 10 days)	306.7 ± 44.2	85.6 ± 33.7
Egg hatch rate (%)	68.8 ± 12.5	17.4 ± 6.9

*(± sd).

was the decrease of the mean egg hatch rate (control: 68.8 ± 12.5%; mutated adults: 17.4 ± 6.9%; $\chi^2 = 9.2$, df = 2, $p = 0.02$).

Effectiveness of the mutants

The level of infestation of cucumbers with the aphid *A. gossypii* was similar in the two greenhouses just before the release of coccinellids (Figure 3). Mutated and control populations showed the same trend in abundance, first a fast and then a slow decrease during the first two days and the following 15 days after the release, respectively (Figure 4A). The decrease in adult populations during the first two days after their release may be due to them having been reared on *E. kuehniella* eggs (Ettifouri and Ferran, 1993). However, the mutated adults were significantly more numerous and remained longer on the plants (23 days) than control adults (10 days). Their egg-laying period was consequently longer (Figure 4B). The low adult fecundity and the weak egg hatch rate accounted for their progeny being less numerous than those of the control (Figure 4C).

Discussion

Non-flying *H. axyridis* were obtained by the use of a chemical mutagen and the selection of adults with wing malformations in successive generations.

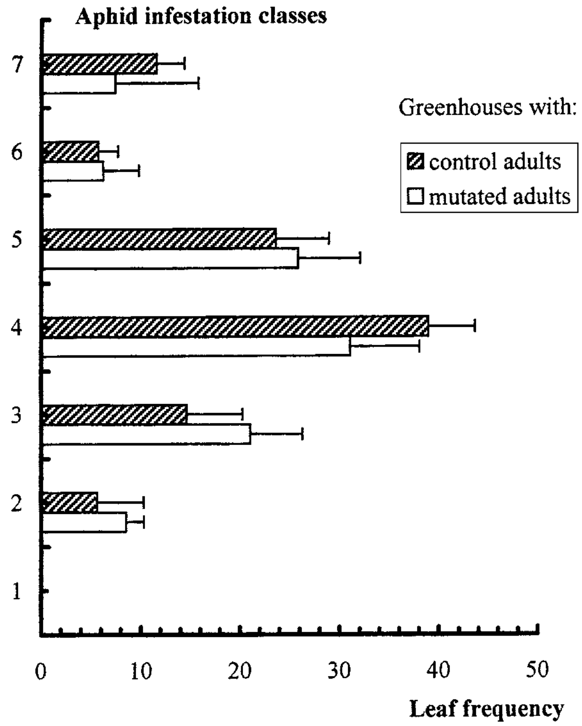

Figure 3. Frequencies of cucumber leaves harbouring aphids in the aphid (*Aphis gossypii*) infestation classes in the two experimental greenhouses before releasing control or mutant adults of *H. axyridis*.

In these adults, the elytra were open and one or both wings were permanently extended. The wings were positioned horizontally or more or less perpendicular to the saggital axis of the body.

The inheritance of wing malformations was not accurately studied (for instance through single pair crosses) due to the reduction of non-flying adult fitness. Nevertheless, this mutant was characterized by some general features. Half of the adults in each generation carry the mutation but have normal elytra and wings, and the other half have abnormal wings. The frequency of these phenotypes did not increase beyond 50% suggesting that homozygosity was not possible. This hypothesis was reinforced by the extinction of line 3 when frequency was increased by inbreeding. The expression of the mutation is temperature dependent. The mutant frequency decreased at temperatures higher than 28 °C. In a control population, wing malformations were observed only when their larvae were reared at temperatures lower than 18 °C. The mutation may raise this thermal threshold above 28 °C so that wing malformation develops when larvae are reared at 20–25 °C.

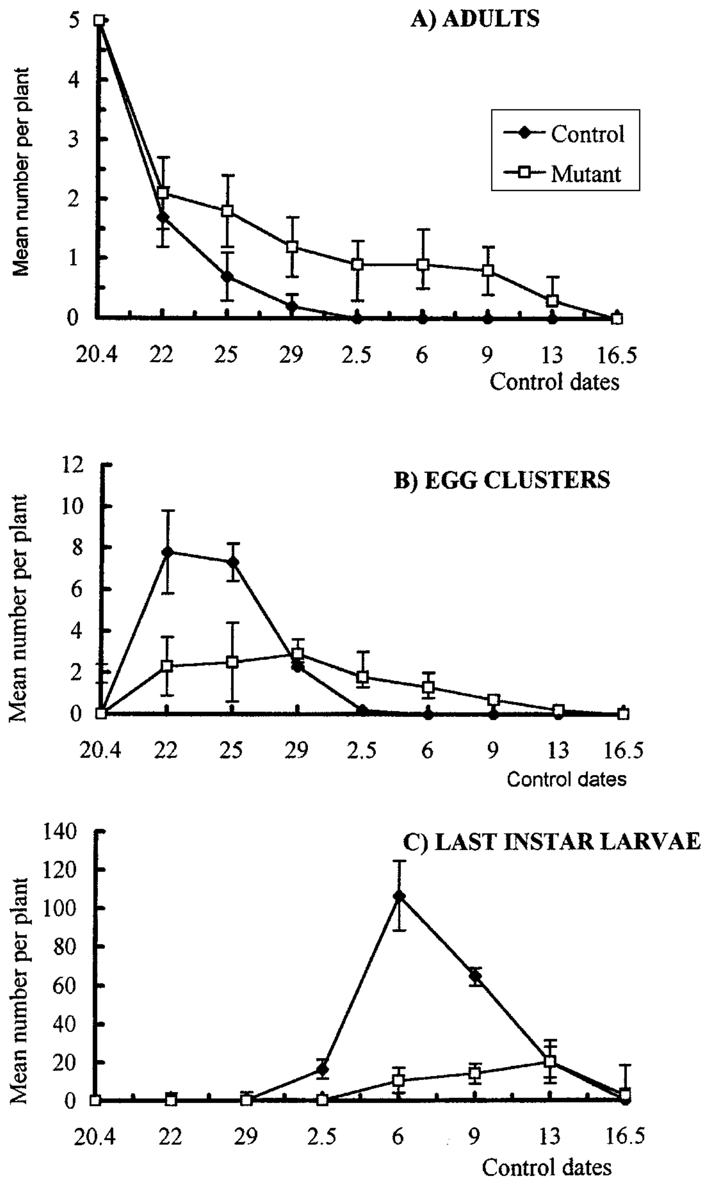

Figure 4. Variation in numbers of *H. axyridis* control and mutant adults, and their progeny in the two cucumber greenhouses infested by the aphid, *A. gossypii*.

Adults with short wings occur in some coccinellid species (Hammond, 1985) and other insects (Roff, 1986). In most of these cases, the genetic basis is polygenic although in some species this trait is controlled by a single gene (Roff, 1986). Marple et al. (1993) showed that adults of *Adalia bipunctata* L. from the wild can be homozygous for a recessive gene that prevents the normal development of the elytra and wings.

The wing malformation in *H. axyridis* was associated with a serious reduction in fitness as in naturally mutated *A. bipunctata* (Marple et al., 1993) Compared to the controls, adult mortality was higher and their fecundity and especially their fertility were lower. This loss of fitness prevents their use in the biological control of aphids. Nevertheless, when the same number of control and mutant adults was released in two greenhouses containing cucumbers infested with the aphid *A. gossypii*, more of the mutant adults remained on the plants and remained for a longer time. The inability to fly seemed to increase the number of adults on the plants.

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