

## Locomotor Behavior of Flightless *Harmonia axyridis* Pallas (Col., Coccinellidae)

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To improve the efficiency of the lady beetle *H. axyridis* as a biological control agent against aphids, a flightless population was obtained by feeding adults with a mutagen and selecting their progeny for nonflying but otherwise morphologically normal individuals. These flightless adults attempted to fly but immediately fell. They softened their fall by opening their elytrae and wings. The inability to fly could result from change in their flying behavior compared to control adults. The flight duration was very much shorter, and the wing beat frequency and, more particularly, the amplitude of the wing beats were clearly lower. More time was spent in the other components of the flight behavior such as wing rotation, wing immobility, and wing folding. The sequence of these patterns differed slightly, due mainly to change in their frequency. The locomotor behavior was not modified by the mutation, which affected only the wing muscles. Searching behavior of mutant adults differed from that of control adults only in that they took longer to encounter and ingest aphids. Nevertheless, the larval growth and reproductive rate remained unchanged. The behavioral and biological features of these flightless adults indicate that it should be possible to use them in biological control programs.

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**KEY WORDS:** *Harmonia axyridis* Pallas; Coccinellidae; mutation; flight; flight behavior; prey search behavior.

### INTRODUCTION

The aphidophagous *Harmonia axyridis* Pallas was introduced into France from China in 1982 and is now mass reared on a substitute prey, the eggs of *Ephestia*

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*kuehniella* Zeller (Lep., Pyralidae). Third-instar larvae reared on this substitute prey are sold for the biological control of the rose aphid, *Macrosiphum rosae* L. (Ferran *et al.*, 1996).

*H. axyridis* in common with aphidophagous ladybirds are good flyers. Adults undertake foraging flights within and between aphid infested field crops and migratory flights in summer to aetivohibernating sites and disperse in spring to wild and cultivated areas (Iperti, 1966; Ferran *et al.*, 1989; Hodek, 1967; Hodek *et al.*, 1993). As the adults are so mobile, only larvae can be used in biological control.

If adults were poorer flyers, they would remain in crops for longer and provide more long-term control (Marple *et al.*, 1993). Brachypterous adults are sometimes observed in natural ladybird populations (Hammond, 1985). These malformations are of either a teratological origin, not transmitted to their progeny, or a consequence of mutations affecting wing morphology (Majerus and Kearns, 1989, Marple *et al.*, 1993). The scarcity of these mutations and their low fitness prevent the mass production of such beetles (Marple *et al.*, 1993).

A flightless population of *H. axyridis* with normal wings has been obtained by feeding adults a chemical mutagen and selecting the flightless adults produced over 17 successive generations. The method and the flightless population itself are now patented (No. 9614859).

This work briefly presents the result of the selection, describes the takeoff and rudimentary flight behavior of these mutants, and assesses the effect of their poor flight ability on their searching behavior.

## MATERIALS AND METHODS

### Selection Method

Young virgin males and females from the control population were fed a mixture of aqueous honey and the chemical mutagen only for 24 h and then fed *E. kuehniella* eggs. Three groups of 200 young adults (males and females) from their progeny were placed on a device kept at a particular set of ambient conditions (patent No. 9614859), which allowed the separation of flightless adults with normal elytrae and wings from those that could fly (first generation). These flightless adults were used to start the second generation. This inbreeding method was then applied till the seventeenth generation. From the twelfth generation some of the previously selected beetles were reared without further selection for five successive generations to test their homozygosity. The results are expressed as the mean percentage of flightless adults. The chi-square test was used to compare the proportion of flying to flightless insects in these two populations.

### Takeoff Behavior

The takeoff behavior of the control and flightless populations (twelfth generation) was compared outdoors on warm sunny days (end of June and July). A  $5 \times 5$ -m area divided into 100 squares ( $0.5 \times 0.5$  m) was delimited by means of string layed out on the ground. A white-painted wooden stick (height, 0.6 m) 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 which 100 adults of each population either landed in or flew over when leaving the area were recorded. The results are expressed as the number landing in each square or flying over a peripheral square on leaving the area.

### Flight Behavior

The flight behavior of 6-day-old male and female beetles (15 individuals per sex) from the control and flightless (twelfth generation) populations was studied in the laboratory. Adults were individually suspended on a stiff plastic string (length, 0.10 m) glued (Super glu 3) to the upper and proximal part of the right elytra. Their body was approximately horizontal and their abdomen oriented toward the lens of the camera. These adults opened their elytrae, extended their wings, and attempted to fly. This behavior was filmed for 180 s with a standard camera (25 frames/s; shutter speed, 1/1000th) and recorded on videotape. The patterns of behavior were observed by running the videotape frame by frame. Wing beat characteristics (frequency and amplitude) were determined by filming 30 other adults (15 males and females), each for two 180-s flight periods. A special camera (600 frames/s) and a control screen were used for this.

The observation of the flight behavior allowed us to define four components: (1) wing beat frequency and amplitude in flight, (2) wings rotated about the longitudinal axis, (3) wings extended but immobile, and (4) wings remain folded under closed elytrae.

The number and duration of these patterns and the frequency (beats/s) and amplitude (degrees) of the wing beats were estimated for both populations and sexes and compared using an *F* test.

For each population, the relationships between the patterns was illustrated using an ethogram. Each pattern was represented by an ellipse whose area was proportional to its average frequency for that population. Each arrow indicates a change between two patterns in terms of the percentage of all coupled patterns in each population. The thickness of the arrow is proportional to the frequency and the head of the arrow indicates the direction of the change. For instance, in the control population, "wings folded" preceded ( $\rightarrow$ ) "wings extended but immobile" in 26.8% of all cases (Fig. 4).

### Searching Behavior

Ladybirds exhibit two types of walking when searching for prey (Nakamuta, 1985; Ferran *et al.*, 1994; Ferran and Dixon, 1996). Extensive search (Bond, 1980) is used to locate patches of prey and is characterized by long linear paths, a fast speed, and few stops. Intensive search (Bond, 1980) is used for patch exploration and follows prey ingestion. It is characterized by sinuous slow paths interrupted by numerous stops. A camera, a videotape recorder, and a computer equipped with a special card (SECAD) allowed us to film individual paths and to calculate the corresponding path parameters.

Adults (33 females and males from each population) were starved for 2 days 10 to 15 days after emergence from pupae. Each adult was then filmed for 1 min in a white arena (height  $h$ , 0.40 m; diameter, 0.50 m) (extensive search). Then each was fed one aphid, *Acyrtosiphon pisum* Harris (weight, 2.0 to 2.20 mg) in a plastic box (area, 6.0 cm<sup>2</sup>), the walls of which were painted with Fluon to prevent aphids and beetles climbing onto the lid. After each beetle finished feeding and resting (and/or grooming), it was filmed for 1 min (intensive search). Control adults which flew out of the arena during filming were discarded. The paths followed by beetles before and after feeding were characterized by the number of stops ( $n/s$ ), the linear speed (mm/s, excluding stop duration), and the angular speed (deg/s).

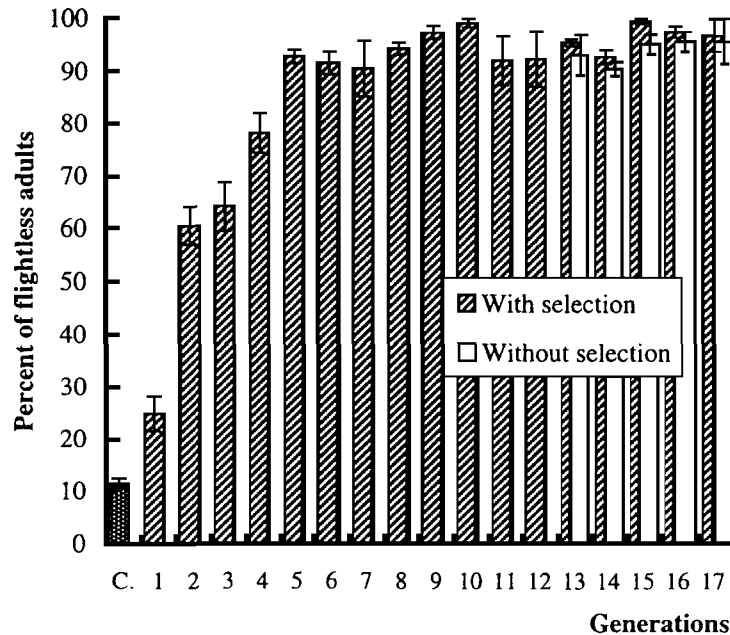
Behavior of adults fed *A. pisum* was characterized by the number of encounters with prey before prey capture by adults ( $n/s$ ), the time (s) that elapsed before prey capture, and the time (s) devoted to ingestion, resting, and/or grooming. Results are expressed as averages plus the corresponding confidence interval ( $P < 0.05$ ). The effect of population, sex, and path type on feeding and locomotor parameters was quantified by mean of variance analysis ( $F$  test).

The last two experiments (flight and searching behavior) were carried out at  $21 \pm 1^\circ\text{C}$ , 70 to 80% relative humidity, and a 16-h day length. Larvae and adults of the two populations were reared on *E. kuehniella* eggs.

## RESULTS

### Selection for Nonflying

In the control population (C), the frequency of adults that did not fly was about 10% (Fig. 1). The frequency of flightless adults in the selected population increased up to the fifth generation, then stabilized between 90 and 100%. As after the twelfth generation there is no difference ( $\chi^2 = 1.9$ ,  $P = 0.8$ ) in the frequency of flightless adults in the selected and nonselected populations, it is likely that all the individuals were homozygous for nonflying in the last five generations.



**Fig. 1.** *Harmonia axyridis* Pallas: selection of a flightless population after ingestion of a chemical mutagen. Hatched, histograms, percentage of flightless adults during selection; white histograms, percentage of flightless adults of the twelfth generation whose progeny were not subjected to selection; C, control population.

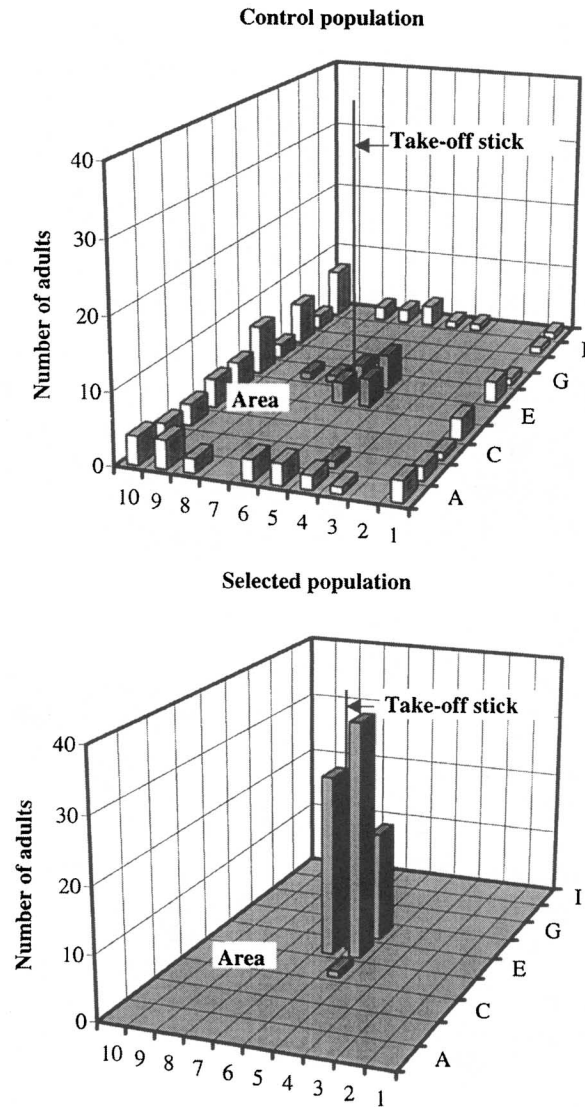
### Takeoff Behavior

Of the 100 control adults that took off from the stick, 19% landed close to the base of the stick and 81% flew out of the area (Fig. 2). All 100 flightless adults from the selected population landed within 0.70 m of the stick. After takeoff, these adults broke their fall by opening their elytrae and extending their wings. That is, the selection had not altered their innate tendency to climb up a vertical stick (positive phototaxis and negative geotaxis), to open their elytrae, and to extend their wings.

### Flight Behavior

In the control and the flightless populations 93.3 and 53.3% of the adults, respectively, showed at least one flight in 180 s. Among control adults, 40% flew during the whole filming period, compared to 0.0% of the flightless adults.

The total number of flight-related behavior patterns observed during the filming period was higher in the flightless population than in the control one



**Fig. 2.** *Harmonia axyridis* Pallas: distance flown by control and flightless adults after their release in an outdoor  $5 \times 5$ -m area divided into 100  $0.5 \times 0.5$ -m squares. Histogram with one white side, number of adults that flew out of the area above the corresponding square; gray histogram, number of adults that landed in the corresponding square.

**Table I.** *Harmonia axyridis* Pallas: Comparison of the Total Number of Flight-Related Behavior Patterns ("Flight," "Wings Rotated," "Wings Extended but Immobile," and "Wings Folded") Observed in 180 s According to Population and Sex

Sex	Population		Average
	Control	Selected	
Female	13.1 ± 18.3 <sup>a</sup>	62.1 ± 30.2	37.6 ± 24.5
Male	20.4 ± 33.4	77.9 ± 27.3	49.1 ± 29.1
Average	16.8 ± 26.5	70.0 ± 25.1	
Comparison	$F = 56.7, P < 0.001$		$F = 2.7, P = 0.10$

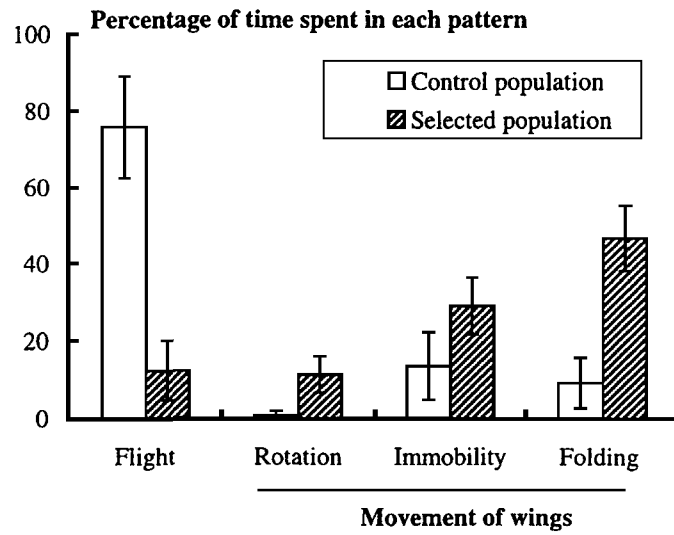
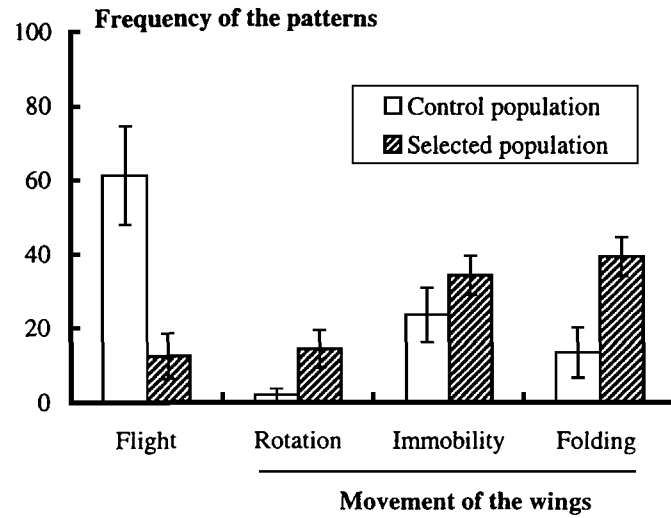
<sup>a</sup>Confidence interval at  $P < 0.05$ .

(Table I). As there was no difference between sexes in this respect, the adults were lumped together for statistical comparisons.

In the control population, the frequency of flight and the percentage of time devoted to it were clearly predominant (Fig. 3). The occurrence (frequency and duration) of the patterns "wings extended but immobile," "wings folded," and "wings rotated" occurred at a decreasing frequency. Flightless adults spent more time in "wings folded," "wings extended but immobile," and "wings rotated." These results are analyzed in detail in Table II. Compared to the control beetles, the flightless ones were characterized by an increase in the number of flights, although the total time spent flying was shorter. The frequency and, particularly, the amplitude of the wing beats were lower. All other patterns were very significantly more numerous and consequently more time-consuming.

Whatever its source, the particular flight behavior of an adult was preceded or followed by one of the three others (Fig. 4). Control adults were characterized by a small number of long-lasting "flights." The three other patterns, particularly "wings rotated" (1.9%), were more rarely adopted. Consequently, the frequency of flight (61.2%) was greater calculated as the percentage of the total number of patterns observed in a given time. In the flightless adults the frequency (calculated as above) of "wings folded" (39.3%) and "wings extended but immobile" (34.1%) was the highest. "Wings rotated" (14.3%) and "flight" (12.3%) were approximately equal in frequency but less than those of the other two behavior patterns.

In the two populations, the most frequent sequence was "wings folded" (resting position) to "wings extended but immobile" (preparation for flight). From "wings folded," control adults either flew immediately (5.1%) or more frequently (26.8%) opened their elytra, extended their wings and then flew (9.0%). "Wings folded" and "wings extended but immobile" rarely preceded "wings rotated" (4.5 and 2.4%, respectively). From "wings folded," flightless adults adopted either "flight" (7.1%), "wings rotated" (10.0%), or "wings extended but immobile" (23.0%). "Wings rotated" preceded more frequently "wings extended



**Fig. 3.** *Harmonia axyridis* Pallas: flight behaviour of control and flightless adults. Flight, wing rotation, immobility, and folding are the four patterns that make up flight behavior.



**Table II.** *Harmonia axyridis* Pallas: Comparison of the Flight Behavior of Control and Mutant Individuals (as the Sexes Do Not Differ, the Averages Are for All Adults)

Parameter	Population		<i>F</i> test
	Control	Selected	
Pattern 1: "Flight"			
Number (in 180s)	3.4 ± 3.6 <sup>a</sup>	8.9 ± 11.6	<i>F</i> = 5.8, <i>P</i> = 0.02
Total duration (s)	136.5 ± 66.6	22.5 ± 38.6	<i>F</i> = 63.5, <i>P</i> < 0.001
Wing beat frequency (beats/s)	76.0 ± 2.9	58.3 ± 1.5	<i>F</i> = 217.2, <i>P</i> < 0.001
Wing beat amplitude (deg)	146.3 ± 12.2	72.6 ± 11.8	<i>F</i> = 127.1, <i>P</i> < 0.001
Pattern 2: "Wings rotated"			
Number (in 180 s)	1.1 ± 3.2	10.7 ± 11.7	<i>F</i> = 18.3, <i>P</i> < 0.002
Total duration (s)	1.7 ± 5.3	20.7 ± 23.1	<i>F</i> = 18.6, <i>P</i> < 0.001
Pattern 3: "Wings extended but immobile"			
Number (in 180 s)	6.4 ± 10.1	22.8 ± 12.8	<i>F</i> = 29.5, <i>P</i> < 0.001
Total duration (s)	24.7 ± 43.4	52.5 ± 36.7	<i>F</i> = 6.9, <i>P</i> < 0.01
Pattern 4: "Wings folded"			
Number (in 180 s)	5.8 ± 12.5	27.6 ± 15.2	<i>F</i> = 35.2, <i>P</i> < 0.001
Total duration (s)	17.0 ± 32.6	84.3 ± 40.8	<i>F</i> = 48.0, <i>P</i> < 0.001

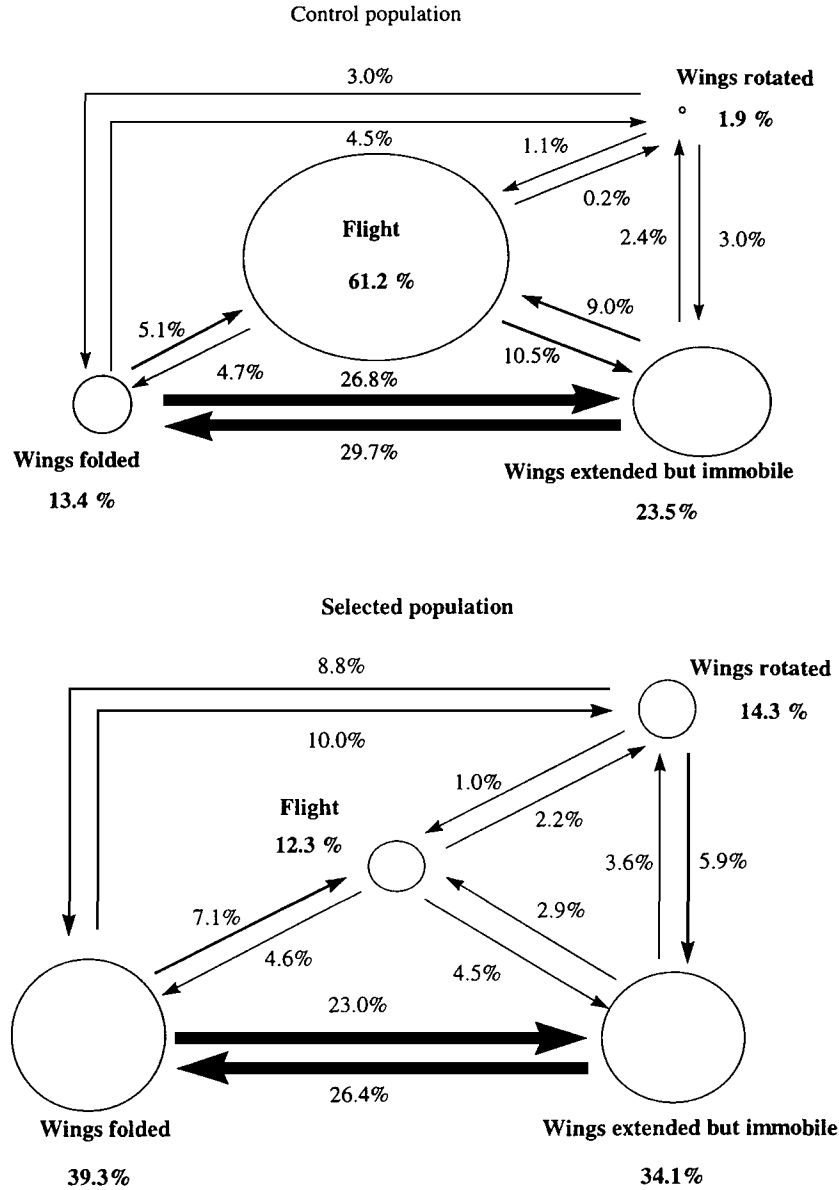
<sup>a</sup>Confidence interval at *P* < 0.05.

but immobile" (5.9%). Whatever the population, the reverse sequences were preferentially used when adults stopped flying.

### Searching Behavior

The behavior of walking adults did not differ significantly between populations (Table III). Irrespective of the population and the search mode (extensive and intensive search), males walked faster than females (linear speed, *F* = 15.3, *P* = 0.001). Prey ingestion resulted in an increase in the number of stops (*F* = 12.4, *P* = 0.0003) and the angular speed (*F* = 14.5, *P* = 0.0003) and a decrease in the linear speed (*F* = 15.4, *P* = 0.0001). This conforms with the well-established behavioral sequence: extensive search, prey ingestion, and intensive search. As the result of an interaction between the factors path and the sex (*F* = 4.2, *P* = 0.04), the linear speed of males was faster than that of females both before and after feeding.

Irrespective of the sex, flightless adults spent more time searching for (*F* = 4.0, *P* = 0.04) and ingesting (*F* = 4.2, *P* = 0.04) prey than control adults (Table IV). The time spent searching for prey was dependent on sex. The males spent more time in searching for (*F* = 10.4, *P* = 0.002) and ingesting (*F* = 14.7, *P* =



**Fig. 4.** *Harmonia axyridis* Pallas: ethogram of the flight behavior of control and flightless adults. Circles, frequency of each pattern; arrows, frequency of the temporal succession between two coupled patterns. These frequencies were calculated from all adults in each population.

**Table III.** *Harmonia axyridis* Pallas: Comparison of Walking Pattern According to Population, Sex, and Before and After Feeding ( $n = 66$  Adults in Each Population; Temperature,  $21 \pm 1^\circ\text{C}$ ; Prey, the Aphid *Acyrtosiphon pisum* Harris)

Factor	Number of stops ( $n/s$ )	Linear speed (mm/s)	Angular speed (deg/s)
Population			
Control	$1.65 \pm 0.25^a$	$17.3 \pm 0.7$	$20.9 \pm 2.1$
Flightless	$1.91 \pm 0.32$	$16.8 \pm 0.7$	$23.5 \pm 2.8$
<i>F</i> test	$F = 1.5$ $P = 0.2$	$F = 0.9$ $P = 0.3$	$F = 2.0$ $P = 0.15$
Sex			
Female	$1.81 \pm 0.31$	$16.0 \pm 0.7$ (b)	$22.4 \pm 2.5$
Male	$1.74 \pm 0.28$	$18.1 \pm 0.8$ (a)	$22.0 \pm 2.5$
<i>F</i> test	$F = 0.1$ $P = 0.7$	$F = 15.3$ $P = 0.001$	$F = 0.1$ $P = 0.8$
Path			
Before feeding	$1.40 \pm 0.23$ (b)	$18.9 \pm 0.7$ (a)	$18.7 \pm 1.7$ (b)
After feeding	$2.15 \pm 0.34$ (a)	$15.2 \pm 0.8$ (b)	$25.6 \pm 3.0$ (a)
<i>F</i> test	$F = 12.4$ $P = 0.0003$	$F = 15.4$ $P = 0.0001$	$F = 14.5$ $P = 0.0003$

<sup>a</sup>Confidence interval at  $P < 0.05$ ; (a) and (b) significant difference between means.

0.003) prey. There were no significant interactions between number of encounters and prey search, feeding, and resting and/or grooming duration.

## DISCUSSION

In aphidophagous ladybirds, most adults capable of flying quickly leave the crops where they have been introduced against aphid pests. To prolong the

**Table IV.** *Harmonia axyridis* Pallas: Comparison of the Searching Behavior Segregated According to Population and Sex ( $n = 66$  Adults in Each Population; Temperature,  $21 \pm 1^\circ\text{C}$ ; Prey, the Aphid *Acyrtosiphon pisum* Harris)

Factor	Number of encounters	Prey search duration (s)	Feeding duration (s)	Resting duration (s)
Population				
Control	$0.01 \pm 0.001^a$	$568 \pm 146$ (b)	$420 \pm 37$ (b)	$521 \pm 140$
Flightless	$0.03 \pm 0.02$	$972 \pm 236$ (a)	$491 \pm 31$ (a)	$800 \pm 152$
<i>F</i> test	$F = 0.8$ $P = 0.37$	$F = 4.0$ $P = 0.04$	$F = 4.2$ $P = 0.04$	$F = 3.5$ $P = 0.06$
Sex				
Female	$0.03 \pm 0.02$	$448 \pm 165$ (b)	$389 \pm 28$ (b)	$663 \pm 191$
Male	$0.01 \pm 0.003$	$992 \pm 353$ (a)	$522 \pm 62$ (a)	$658 \pm 221$
<i>F</i> test	$F = 2.1$ $P = 0.14$	$F = 10.4$ $P = 0.002$	$F = 14.7$ $P = 0.003$	$F = 0.1$ $P = 0.97$

<sup>a</sup>Confidence interval at  $P < 0.05$ ; (a) and (b) significant difference between means.

residence time of adults in crops, a homozygous flightless *Harmonia axyridis* was selected. When they attempted to fly, the flightless landed close to their point of taking-off. They softened their landing by opening their elytrae and wings.

In lady beetles, flight behavior consist of four patterns: flight in which the wings beat with a frequency and the amplitude, "wings rotated" about their longitudinal axis, "wings extended but immobile," and "wings folded" under closed elytrae. All these patterns were observed in control and flightless adults. Nevertheless, the flightless adults made more attempted flights in a given time but spent far less time flying. In addition, the frequency and, particularly, the amplitude of their wing beats were lower. The three other patterns were observed more frequently, especially "wings folded" and "wings extended but immobile."

The flight sequence in the two populations is as follows: from "wings folding," which was probably a resting position, control adults prepared their flight by opening their elytrae and wings and then mainly flew. From "wings folding," the flightless adults adopted preferentially either "wings extended but immobile" and then "wings rotated" or, less frequently, "wings rotated" and then "wings extended but immobile." "Flight" progressed mainly to "wings folded" and, at a lower frequency, to "wings extended but immobile." Whatever the population, the reverse sequences were preferentially used when adults stopped flying.

This is associated with anatomical anomalies in the wing muscles (Tourniaire, personal communication). As an *Drosophila melanogaster* L., the mutation may have affected one or several genes, controlling muscle development through ecdysone production during metamorphosis (Sandstrom *et al.*, 1997), encoding for proteins that participate in wing muscle organization, e.g., tropomyosin (Kreuz *et al.*, 1996) and actin (Sparrow *et al.*, 1991), or enzymes involved in energy metabolism, e.g.,  $\alpha$ -glycerophosphate deshydrogenase (O'Brien and Macintyre, 1972).

The locomotor behavior of flightless and normal adults, before and after prey ingestion, was similar. The mutation affected only wing muscles. Foraging for prey was slightly affected by mutation. Flightless adults spent more time foraging for and ingesting prey. This feature was insignificant because larval growth and reproductive rate remained unchanged (Ferran *et al.*, 1998).

From an applied point of view, the inability to fly enhances the probability of adults remaining in a crop. As they fall from plants when they attempt to fly, they need to be able to relocate plant. Many coccinellid adults visually orient to plants and prey (Nakamuta, 1985; Khan Khalil *et al.*, 1985; Obata, 1997; Hatting and Samways, 1995; Lambin *et al.*, 1995).

The behavioral and biological features of these adults are such that it should be possible shortly to use this flightless population in biological control programs.

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