Locomotory behavior of flightless *Harmonia axyridis* Pallas (Col., Coccinellidae)

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Abstract

To improve the efficiency of the ladybeetle *Harmonia axyridis* as a biological control agent against aphids, a flightless population was produced by feeding adults with a mutagen and selecting their progeny for non-flying but otherwise morphologically normal individuals. The inability to fly could result from a change in their flying behavior compared with control adults. Flight duration was very much shorter, wing beat frequency and more particularly the amplitude of the wing beats were clearly lower. More time was spent on the other components of flight behavior such as wing rotation, wing immobility and wing folding. The sequence of these patterns differed slightly mainly due to changes in their frequency. The locomotory behavior was not modified by the mutation, which only affected the wing muscles. The searching behavior of mutant adults differed from that of control adults only in that they took longer to encounter and ingest aphids. As this difference is not important, it should be possible to use this flightless population in biological control programs. © 2000 Elsevier Science Ltd. All rights reserved.

Keywords: Harmonia axyridis; Mutation; Flight behavior; Prey search behavior

1. Introduction

The aphidophagous ladybird *Harmonia axyridis* Pallas was introduced into France from China in 1982 and has been mass reared on a substitute prey, the eggs of *Ephestia kuehniella* Zeller (Lep., Pyralidae). Third instar larvae reared on this substitute prey are sold for biological control of the rose aphid, *Macrosiphum rosae* L. (Ferran et al., 1996).

H. axyridis, in common with some other aphidophagous ladybirds, is a good flyer. Adults undertake foraging flights within and between aphid-infested field crops, migratory flights in summer to aestivo-hibernating sites, and disperse in spring to wild and cultivated areas (Iperti, 1966; Ferran et al., 1989; Hodek, 1967; Hodek et al., 1993; Hodek and Honek, 1996; Obata, 1986). As the adults are so mobile, only the larvae can be used in biological control.

If adults were poorer flyers they would remain on 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.

This work presents the results of the selection, describes the take-off and rudimentary flight behavior of these mutants, and assesses the effect of their poor flight ability on their searching behavior.

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2. Materials and methods

2.1. Method of selection

Young virgin males and females from the control (normal) population were fed a mixture of aqueous honey and the chemical mutagen for 24 h only and then fed *E. kuehniella* eggs (patented method, no. 9614859). The selection procedure involved placing three groups of 200 young adults from each successive generation on a device kept at a particular set of ambient conditions (patented method). The adults with normal elytra and wings that did not fly, were used to start the subsequent generations. From the twelfth generation some of the previously selected beetles were reared without further selection for five successive generations to test their homozygozity. The results are expressed as the mean percentage of flightless adults. A 'chi-square' test was used to compare the proportion of flying to flightless insects in these two populations.

2.2. Take-off behavior

The take-off behavior of the control and flightless populations 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 delimited by means of string laid 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 in which 100 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 a peripheral square on leaving the area.

2.3. 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 elytron. As soon as they are suspended, adults opened their elytra and wings and started (or attempted) to fly. Their body was approximately horizontal and their abdomen oriented toward the lens of the camera. The adults opened their elytra, 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 video tape. The patterns of behavior were observed by running the video tape frame by frame. Wing beat characteristics (frequency and amplitude) were determined by filming 30 other adults (15 males and 15

females), each for two 180 s flight periods. A special camera (600 frames/s) and control screen were used for this.

The observations on the flight behavior allowed us to define four patterns: 1 — wing beat frequency and amplitude in flight, 2 — wings rotated about the longitudinal axis, 3 — wings extended but immobile and 4 — wings remained folded under closed elytra.

The number and duration of these patterns were estimated for both populations and sexes and compared by using an 'F' test.

2.4. Searching behavior

Ladybirds exhibit two types of walking when searching for prey (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. After prey ingestion, the prey patch is explored by intensive search (Bond, 1980). Intensive search is characterized by sinuous paths and slow movement interrupted by numerous stops. A camera, a video tape recorder and a computer equipped with a special card allowed us to film individual paths and to calculate the corresponding path parameters.

Adults (33 females and 33 males from each population) were starved for two days, 10 to 15 days after emergence from pupae. Each adult was then filmed for 1 min in a white arena (height, 0.40 m; diameter, 0.50 m) (extensive search). Then each was fed one aphid, *Acyrthosiphon pisum* Harris (weight, 2.0 mg to 2.20 mg), in a plastic box (area, 6.0 cm^2) the walls of which were painted with Fluon to prevent aphids and beetles climbing on to the lid. After each beetle finished feeding and resting (and/or grooming), it was filmed for 1 min (intensive search). Control adults that 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 index (°/s).

The behavior of adults fed *A. pisum* was characterized by the number of encounters with prey before prey capture (n/s), the time (s) that elapsed before prey capture, and the time (s) devoted to ingestion, resting and/or grooming.

Results were expressed as averages plus the corresponding confidence interval ($p<0.05$). The effect of population, sex and path type on feeding and locomotory parameters was quantified by means of variance analysis ('F' test).

The last two experiments (flight and searching behavior) were carried out at 21±1°C, with 70 to 80% relative humidity and a 16 h day length. Larvae and adults of the two populations were reared on *E. kuehniella* eggs.

3. Results

3.1. Selection for non-flying

In the control population (C), the frequency of adults that did not fly was about 10% (Fig. 1). The incidence of flightless adults in the selected population increased up to the fifth generation then stabilized between 90% and 100%. As after the 12th generation there is no difference ($\chi^2=1.9$; $p=0.8$) in the incidence of flightless adults in the selected and non-selected populations, it is likely that all the individuals were homozygous for non-flying in the last five generations.

3.2. Take-off 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 of the 100 adults from the selected population landed within 0.70 m of the stick.

3.3. Flight behavior

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

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 with

Control population

Selected population

Fig. 2. Distance flown by control and flightless adults of *H. axyridis* after their release in an outdoor 5 m×5 m area divided into 100 0.5 m×0.5 m squares (columns with one white side, number of adults that flew out of the area above the corresponding square; gray columns, number of adults that landed in the corresponding square).
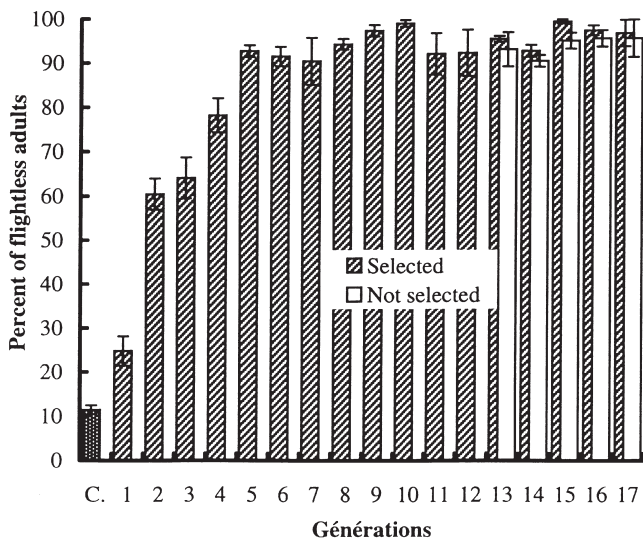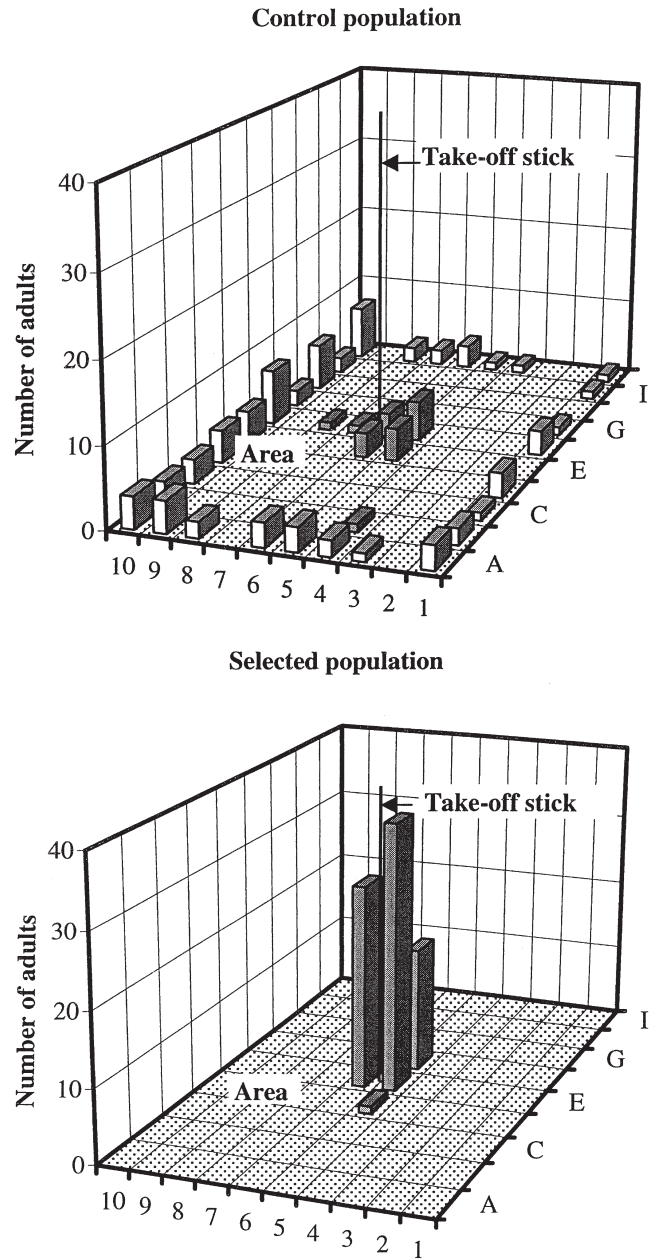

Fig. 1. Selection of a flightless population of *H. axyridis* after ingestion of a mutagenic chemical (shaded columns, percentage of flightless adults during selection; open columns, percentage of flightless adults of the twelfth generation whose progeny was not subjected to selection until the seventeenth generation; C, control population).

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 1. Compared with 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.
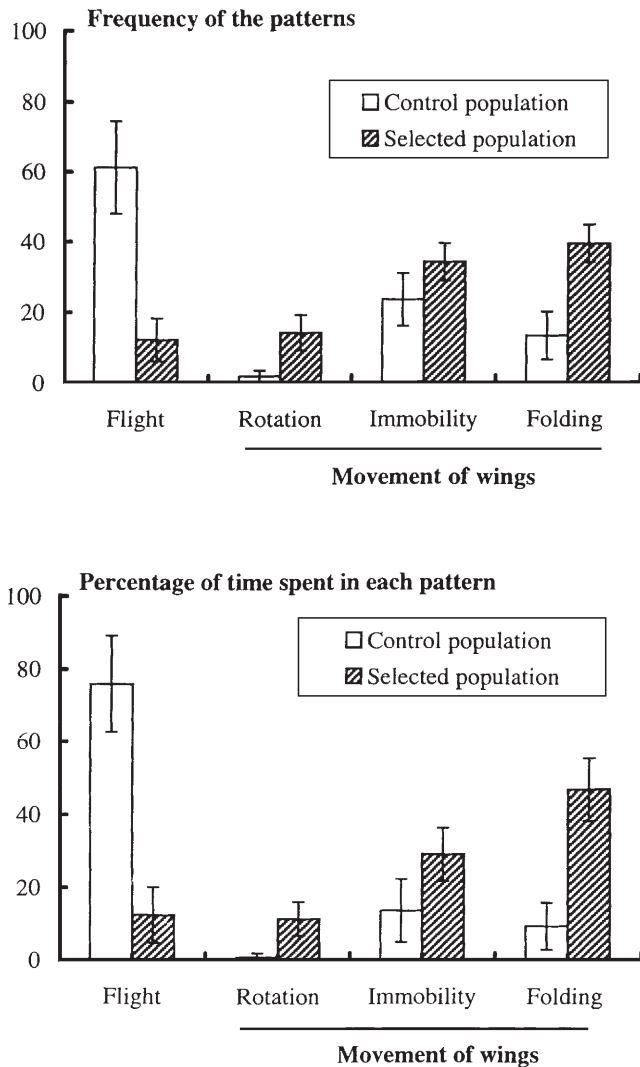
Fig. 3. Flight behaviour of control and flightless adults of *H. axyridis* (flight, wing rotation, immobility and folding were the four patterns that make up the flight behaviour).

3.4. Searching behavior

The behavior of walking adults did not differ significantly between populations (Table 2). Prey ingestion resulted in an increase in the number of stops ($F=12.4$; $p=0.0003$) and 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. Irrespective of the population and the search mode (extensive and intensive search), males walked faster than females (linear speed, $F=15.3$; $p=0.001$). As a result of an interaction between the factors path and linear speed, the linear speed of males was faster than that of females both before and after feeding ($F=4.2$; $p=0.04$).

Irrespective of the sex, flightless adults spent more time searching for ($F=4.0$; $p=0.04$) and ingesting prey ($F=4.2$; $p=0.04$) than control adults (Table 3). 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 prey ($F=14.7$; $p=0.003$). There were no significant interactions between number of encounters, prey search, feeding and resting duration.

4. Discussion

In aphidophagous ladybirds, most adults capable of flying quickly leave crops where they have been introduced against aphid pests. In order to prolong the residence time of adults on crops, a homozygous flightless *Harmonia axyridis* was selected.

When they attempted to fly, the flightless adults landed close to their point of take-off. They softened their landing by opening their elytra and 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 elytra and to extend their wings.

In ladybeetles, flight behavior consists of four patterns: flight in which the wings beat with a frequency and amplitude, 'wing rotated' about their longitudinal axis, 'wings extended but immobile' and 'wings folded' under closed elytra. All these patterns were observed in both control and flightless adults. Nevertheless, the flightless adults made more attempts to fly 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'.

This is associated with anatomical anomalies in the wing muscles (Tourniaire, personal communication). As in *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 organisation (e.g., tropomyosin, Kreuz et al., 1996; and actin, Sparrow et al., 1991) or enzymes involved in energy metabolism (e.g., the α-glycerophosphate dehydrogenase, O'Brien and Macintyre, 1972).

The locomotory behavior of flightless and normal adults, before and after prey ingestion, was similar. Foraging for prey was only slightly affected by the mutation. Flightless adults spent more time foraging for and ingesting prey. The effect of this difference on *H. axyridis* biology 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 on crops. As they fall from plants when they attempt to fly, they need to be able to relocate plants. Many coccinellid

Table 1
H. axyridis—Comparison of the flight behavior of control and mutant *H. axyridis* individuals (as the sexes do not differ, the average was for all adults)

Parameter	Population		*F* test
	Control	Selected	
Pattern 1: 'Flight'			
Number (in 180 s)	3.4±3.6[a]	8.9±1.6	$F=5.8, p=0.02$
Total duration (s)	136.5±66.6	22.5±38.6	$F=63.5, p<0.001$
Wing beat frequency (beats/s)	76.0±2.9	58.3±1.5	$F=217.2, p<0.001$
Wing beat amplitude (°)	146.3±12.2	72.6±11.8	$F=127.1, p<0.001$
Pattern 2: 'Wings rotated'			
Number (in 180 s)	1.1±3.2	10.7±11.7	$F=18.3, p<0.002$
Total duration (s)	1.7±5.3	20.7±23.1	$F=18.6, p<0.001$
Pattern 3: 'Wings extended but immobile'			
Number (in 180 s)	6.4±10.1	22.8±12.8	$F=29.5, p<0.001$
Total duration (s)	24.7±43.4	52.5±36.7	$F=6.9, p<0.01$
Pattern 4: 'Wings folded'			
Number (in 180 s)	5.8±12.5	27.6±15.2	$F=35.2, p<0.001$
Total duration (s)	17.0±32.6	84.3±40.8	$F=48.0, p<0.001$

[a] Confidence interval at $p<0.05$.

Table 2
H. axyridis — comparison of walking pattern according to population, sex and before and after feeding ($n=66$ adults in each population; temperature, 21±1°C; prey, the aphid *Acyrthosiphon pisum* Harris)

Factor	*H. axyridis*	Number of stops (n/s)	Linear speed (mm/s)	Angular index (°/s)
Population	Control	1.65±0.25[a]	17.3±0.7	20.9±2.1
	Flightless	1.91±0.32	16.8±0.7	23.5±2.8
	F test	$F=1.5$	$F=0.9$	$F=2.0$
		$p=0.2$	$p=0.3$	$p=0.15$
Sex	Female	1.81±0.31	16.0±0.7[c]	22.4±2.5
	Male	1.74±0.28	18.1±0.8[b]	22.0±2.5
	F test	$F=0.1$	$F=15.3$	$F=0.1$
		$p=0.7$	$p=0.001$	$p=0.8$
Path	Before feeding	1.40±0.23[c]	18.9±0.7[b]	18.7±1.7[c]
	After feeding	2.15±0.34[b]	15.2±0.8[c]	25.6±3.0[b]
	F test	$F=12.4$	$F=15.4$	$F=14.5$
		$p=0.0003$	$p=0.0001$	$p=0.0003$

[a] Confidence interval at $p<0.05$.
[b] Significant difference between means.
[c] Significant difference between means.

Table 3
H. axyridis — comparison of the searching behavior segregated according to population and sex ($n=66$ adults in each population; temperature, 21±1°C; prey, the aphid *Acyrthosiphon pisum* Harris)

Factor	*H. axyridis*	Number of encounters	Prey search duration (s)	Feeding duration (s)	Resting duration (s)
Polulation	Control	0.01±0.001[a]	568±146[c]	420±37[c]	521±140
	Flightless	0.03±0.02	972±236[b]	491±31[b]	800±152
	F test	$F=0.8$	$F=4.0$	$F=4.2$	$F=3.5$
		$p=0.37$	$p=0.04$	$p=0.04$	$p=0.06$
Sex	Female	0.03±0.02	448±165[c]	389±28[c]	663±191
	Male	0.01±0.003	992±353[b]	522±62[b]	658±221
	F test	$F=2.1$	$F=10.4$	$F=14.7$	$F=0.1$
		$p=0.14$	$p=0.002$	$p=0.003$	$p=0.97$

[a] Confidence interval at $p<0.05$.
[b] Significant difference between means.
[c] Significant difference between means.

adults visually orientate 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 to use this flightless population shortly in biological control programs.

Acknowledgements

References

Bond, A.B., 1980. Optimal foraging in a uniform habitat: the search mechanism of the green lacewing. Anim. Behav. 28, 10–19.

Ferran, A., Dixon, A.F.G., 1996. Foraging behaviour of ladybird larvae (Col., Coccinellidae). Eur. J. Entomol. 90, 383–402.

Ferran, A., Gubanti, P., Iperti, G., Migeon, A., Onillon, J., 1989. La répartition spatiale des différents stades de *Coccinella septempunctata* L. dans un champ de blé: variation au cours de la saison. Entomol. Exp. Appl. 53, 229–236.

Ferran, A., Ettifouri, M., Clement, P., Bell, W.J., 1994. Sources of variability in the transition from extensive to intensive search in coccinellid predators (Coleoptera: coccinellidae). J. Insect Behav. 7, 633–647.

Ferran, A., Nikam, H., Kabiri, F., Picart, J.L., De Herce, C., Brun, J., Iperti, G., Lapchin, L., 1996. The use of *Harmonia axyridis* larvae (Col., Coccinellidae) against *Macrosiphum rosae* (Hemiptera, Aphidae) on rose bushes. Eur. J. Entomol. 93, 59–67.

Ferran, A., Brun, J., Tourniaire, R., Giuge, L., Gambier, J., 1998. Sélection et caractérisation d'une souche de la coccinelle *Harmonia axyridis* (Pallas) inacapable de voler. In: 1er Colloque Transnational sur les Luttes Biologique, Intégrée et Raisonnée, Lille, 21–23 January., pp. 529–535.

Hammond, P., 1985. Dimorphism of wings, wing folding and wing toiletry devices in the ladybird *Rhyzobius litura* (F.) (Col., Coccinellidae) with discussion of interpopulation variation in this and other wing-dimorphic beetle species. Biol. J. Linn. Soc. 24, 15–33.

Hatting, V., Samways, M.J., 1995. Visual and olfactory location of biotopes, prey patches and individual prey by the ladybeetle *Chilocorus nigritus*. Entomol. Exp. Appl. 75, 87–98.

Hodek, I., 1967. Bionomics and ecology of predaceous Coccinellidae. Ann. Rev. Entomol. 12, 79–104.

Hodek, I., Honek, A., 1996. Ecology of Coccinellidae. Kluwer Academic Publishers, Dordrecht.

Hodek, I., Iperti, G., Hodkova, M., 1993. Long-distance flights in Coccinellidae (Coleoptera). Eur. J. Entomol. 90, 403–414.

Iperti, I., 1966. Ecobiologie des coccinelles aphidiphages: les migration. Colloque de l'INRA 36, 107–120.

Khan Khalil, S.M.A., Shah, Baloch, U.K., 1985. Optical orientation in predatory coccinellids. Pakistan J. Agric. Res. 6, 40–44.

Kreuz, A.J., Simcox, A., Maughan, D., 1996. Alterations in flight muscle ultrastructure and function in *Drosophila* tropomyosin mutants. J. Cell Biol. 135, 673–687.

Lambin, M., Ferran, A., Maugan, K., 1995. La prise d'informations sensorielles chez la coccinelle *Harmonia axyridis*. Entomol. Exp. Appl. 79, 121–130.

Majerus, M.O., Kearns, P., 1989. Ladybirds. Richmond Publishing, Slough.

Marple, N.M., De Jong, P.W., Ottenheim, M.M., Verhoog, M.D., Brakefield, P.M., 1993. The inheritance of a wingless character in the 2 spot ladybird (*Adalia bipunctata*). Entomol. Exp. Appl. 69, 69–73.

Nakamuta, K., 1985. Mechanism of the switch over from extensive to area-concentrated search behavior of the ladybeetle *Coccinella septempunctata bruckii*. J. Insect Physiol. 31, 849–856.

Obata, S., 1986. Determination of hibernating site in the ladybird beetle *Harmonia axyridis* Pallas (Col., Coccinellidae). Kontyû, Tokyo 54, 218–223.

Obata, S., 1997. The influence of aphids on the behaviour of adults of the ladybird beetle *Harmonia axyridis* (Col., Coccinellidae). Entomophaga 42, 103–106.

O'Brien, S.J., Macintyre, R.J., 1972. The α-glycerophosphate in *Drosophila melanogaster*. II — genetic aspects. Genetics 71, 127–138.

Sandstrom, D.J., Bayer, C.A., Fristom, J.W., Restifo, L.L., 1997. Broad complex transcription factors regulate thoracic muscle attachment in Drosophila. Develop. Biol. 181, 168–185.

Sparrow, J., Reedy, M., Ball, E., Kyrtatas, V., Molloy, J., Durston, J., Hennessy, E., White, D., 1991. Functional and ultrastructure effects of a missense mutation in the indirect flight muscle-specific actin gene of *Drosophila melanogaster*. J. Mol. Biol. 222, 963–982.