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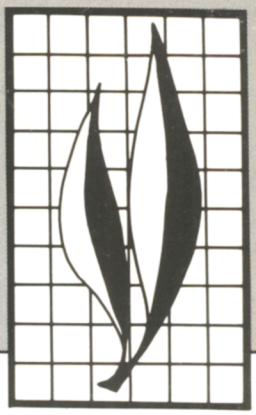

Activity Levels of Genetically Manipulated and Wild Strains of *Metaseiulus occidentalis* (Nesbitt) (Acarina: Phytoseiidae) Compared as a Method to Assay Quality

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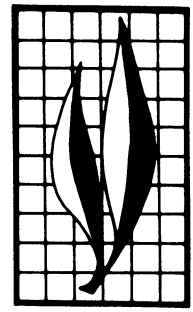

ABSTRACT

The activity levels (AL) of males and females of four genetically manipulated strains and one wild strain of the phytoseiid *Metaseiulus occidentalis* (Nesbitt) were measured using a computerized video-tracking system. Such AL measurements offer the potential for a quantitative laboratory technique to evaluate the relative quality of strains. Adult females of a Permethrin-Organophosphorous-resistant (P-R) strain consistently had an average AL significantly lower than those of the Wild strain and of the three other laboratory strains. The low activity of the P-R strain is unlikely to be due to decreased strain quality associated with laboratory rearing procedures per se, because the three other long-established laboratory strains exibited AL averages comparable to the Wild strain. Adult males exibited no differences in AL. Possible reasons for the lower AL of the P-R strain are discussed.

Hungry adult females tested for AL exhibited three types of running patterns: edge, circle, and nonspecific. The Wild females ran mostly in circles, whereas the P-R females ran mostly near the edge. The males of the P-R and Wild strains did not run in circles, and displayed nearly equal proportions of the edge and nonspecific patterns. Pattern type appears to be related to rate of movement.

A genetic analysis of AL was conducted using the Wild and P-R strains. The AL measurements of reciprocal F1 and F2 females indicated that the high AL of the Wild strain is dominant over the low AL of the P-R strain. Attempts to repeat the F1 and F2 tests were unsuccessful because of a previously undetected, one-way mating incompatibility between the reciprocal crosses; however, the data indicate that activity level is a heritable trait.

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Activity Levels of Genetically Manipulated and Wild Strains of *Metaseiulus occidentalis* (Nesbitt) (Acarina:Phytoseiidae) Compared as a Method to Assay Quality[1]

INTRODUCTION

IN THE LAST SIX years, genetically altered phytoseiid predators (Acari:Phytoseiidae) have been used in integrated pest management systems to control spider mites (Acari: Tetranychidae) in orchards and vineyards (for a review see Hoy 1985). Four species of predatory phytoseiids have been selected artificially for resistance to insecticides (Croft and Meyer 1973; Hoy and Knop 1981; Markwick 1986; Roush and Hoy 1981a; Strickler and Croft 1982; Schulten and van de Klashorst 1979).

A colony of *Metaseiulus* (= *Typhlodromus or Galendromus*) *occidentalis* (Nesbitt), which had previously acquired a high level of resistance to organophosphorous insecticides (OP) (Hoy et al. 1979; Hoy and Knop 1981; Hoyt 1969a) and to sulfur (Hoy and Standow 1982) in the field, was artificially selected using carbaryl and sulfur (Hoy and Standow 1981; Roush and Hoy 1981a) to develop a Carbaryl-OP-Sulfur-resistant (COS-R) strain (Hoy 1984), and using permethrin to develop a Permethrin-OP-resistant (P-R) strain (Hoy and Knop 1981). Field tests of these laboratory-selected *M. occidentalis* strains showed that the P-R strain and the COS-R strain could establish in the field, overwinter, disperse, and survive pesticide applications (Hoy 1982; Hoy, Castro, and Cahn 1982; Hoy, Knop, and Joos 1980; Roush and Hoy 1981b). The COS-R strain has been successful as a genetically manipulated biological control agent; it is now mass-produced and commercially available in California (Headley and Hoy 1986; Hoy 1985; Hoy, Castro, and Cahn 1982).

The P-R strain, though, has been less successful. Two of its disadvantages are that (1) it can tolerate only low levels of permethrin in the field and (2) reproductive isolation from native populations is necessary to maintain the polygenically determined resistance (Hoy and Knop 1981; Hoy, Westigard, and Hoyt 1983). Other factors also may affect the efficacy of the P-R strain as a predator in the field. Hoy, Westigard, and Hoyt (1983) suggested that the P-R strain may disperse more slowly than the COS-R strain. Hoy and Cave (1985) also found that adult reproductive pairs of the P-R strain took longer to make initial contact and spent more time in copulation than three other laboratory-maintained strains of *M. occidentalis* not selected for pesticide resistance. Hoy and Cave suggested that the P-R strain might have a lower level of activity, due either to laboratory conditioning or inadvertent selection for a less active strain during the selection for permethrin resistance.

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During the selection for permethrin resistance, Hoy and Knop (1981) observed that large numbers of *M. occidentalis* females ran off leaf disks treated with permethrin. Runoff does not occur when *M. occidentalis* are treated with carbaryl, organophosphorous insecticides, or sulfur (M. A. Hoy, unpublished). These runoff mites may represent a gene pool that was inadvertently and consistently selected out of the P-R population during selection for permethrin resistance. Such selection could affect the performance of the P-R strain in the field.

The quality of mass-reared or artificially selected arthropods is of particular concern (Boller 1972; Mackauer 1976). During propagation in insectaries, fitness may be reduced as a result of acclimatization to laboratory conditions or genetic processes, which include random genetic drift, founder effects, inbreeding, and selection (Bush and Hoy 1984; Joslyn 1984; Mackauer 1980). The phenotypic alterations associated with the environment are usually reversible; those changes at the genetic level can usually be corrected only by means of special breeding programs (Mackauer 1980). Alterations in genotype or phenotype of laboratory-reared arthropods have been documented (Berlocher and Friedman 1981; Bush 1978; Raulston 1975). How such changes affect the performance of laboratory-reared or genetically manipulated strains under natural conditions has become an issue of major importance in the field of insect rearing (King and Leppla 1984).

Relatively fast and valid laboratory methods are necessary to assay the "quality" of laboratory-propagated arthropods (Boller 1978; Chambers 1977). It is important to be able to detect genetic, physiological, and behavioral deviations of laboratory strains from wild populations before their release into the field. Once in the field, the released strain is difficult to monitor, and therefore problems are difficult to identify and correct. Dr. F. Bigler (Swiss Federal Research Station for Agronomy, CH-8046 Zurich, unpublished) postulated that laboratory measurements of locomotion could function as an index of the field efficacy of another biological control agent, *Trichogramma.* He utilized a different computer-based technique to measure locomotor activity. Bigler was able to increase activity of one *Trichogramma* strain through artificial selection, and he subsequently documented enhanced field efficacy. In addition, he found that the locomotor activity of females from different strains of *T. maidis* Pintureau and Voegele were positively correlated with the potential for parasitization in the field (Bigler and Bieri, in press).

We hypothesize that the activity level of a predatory mite would have a direct effect on traits such as dispersal, the search for mates and prey, and the ability to overcome prey. All of these characteristics seem to be important in determining the success of a population in the field. We suggest that a laboratory-determined measurement of activity level has the potential of functioning as a predictor of field efficacy of some laboratory-reared nonflying arthropod predators.

The goals of this study were (1) to develop and evaluate a method for measuring the activity level of *M. occidentalis,* under controlled laboratory conditions, using a video camera microcomputer tracking system developed by Hoy, Globus, and Norman (1983); (2) to determine whether differences in rate of locomotion exist between the P-R strain, other laboratory-maintained strains, and a wild strain; and (3) to determine whether activity level (AL) is heritable and, if so, the mode of inheritance involved.

MATERIALS AND METHODS

Organisms and Equipment Used

Colony source

Four genetically improved strains of *M. occidentalis* (Hoy 1984) and a freshly collected wild strain were used in this study.

Permethrin-OP-resistant strain (P-R). The P-R strain was initiated in November 1977 from 200 to 300 diapausing adult females taken from bark chips collected in an apple orchard in Wenatchee, Washington (Hoy and Knop 1981). The most recent selection (#38) with permethrin was made on January 24, 1985. The P-R strain had acquired resistance to organophosphorous insecticides (OPs) in the field before laboratory selection with permethrin began (Hoyt 1969b). This strain's permethrin resistance is polygenically inherited (Hoy and Knop 1981).

Permethrin-OP-resistant field-recovery strain (PF-R). On June 4, 1980, after the eighteenth selection with permethrin, the P-R strain was released into a Washington apple orchard. Subsequent to release, low-dose permethrin treatments were made regularly to continue selection pressure (Hoy, Westigard, and Hoyt 1983). On October 17, 1984, S. C. Hoyt recovered some specimens of *M. occidentalis* from cloth tree bands and bark samples taken from the same orchard. Forty-two diapausing (fertilized) females from those samples started the PF-R strain at the University of California, Berkeley. Before testing began, the PF-R and P-R strains were screened simultaneously with 2.0 g ai/100 L water, and 44 percent and 74 percent survived, respectively (Mueller-Beilschmidt 1986). The PF-R strain has not been treated with permethrin since recovery from the field (8 months).

Carbaryl-OP-Sulfur-resistant strain (COS-R). The COS-R strain was selected for carbaryl and sulfur resistances in the laboratory (Hoy 1984; Hoy and Standow 1982; Roush 1979; Roush and Hoy 1981a, b). The OP-resistance trait had been acquired naturally in the field, as by many populations of *M. occidentalis* found in agricultural areas of California (Hoy et al. 1979; Hoy and Knop 1979; Huffaker and Kennett 1953). The last (twelfth) selections, made before testing began in April 1985, were with sulfur on February 27 and 28, and with carbaryl on March 6, 1985. Carbaryl and sulfur resistances are determined by a single incompletely dominant gene each (Hoy and Standow 1982; Roush and Hoy 1981a).

Carbaryl-OP-Permethrin-resistant strain (COP-R). The COP-R strain was started in August 1980 by crossing 75 Carbaryl-OP-resistant males with 175 Permethrin-OP-resistant females (Hoy 1984). The COP-R strain was screened with 2.4 g ai carbaryl/L and 2.0 g ai permethrin/100 L water immediately before these tests; survival in the two tests was 58 percent and 64 percent, respectively (Mueller-Beilschmidt 1986). The last (eleventh) selections were made using permethrin on February 27, 1984, and carbaryl on March 2, 1984.

Wild strain (W). The Wild strain was collected from almond trees west of Modesto, California on April 17, 1985. The colony was started with 85 females and 5 males. The species was confirmed by slide mounting adult females in Hoyer's mounting medium and using a key from Schuster and Pritchard (1963).

The Wild strain showed no substantial resistance to either carbaryl (LD_{50} = 0.05 g ai/L water) or permethrin (LD_{50} = 0.12 g ai/100 L) (Mueller-Beilschmidt 1986), but it most likely has some resistance to organophosphorous insecticides.

Colony maintenance

The colonies were reared separately on approximately 7.5 × 7.5 cm waxed, black construction paper resting on water-soaked cotton in plastic trays. Predators were fed four times weekly with all stages of live two-spotted spider mites, *Tetranychus urticae* Koch. Spider mites were reared in a greenhouse on pinto beans, *Phaseolus vulgaris* (L.). *Metaseiulus occidentalis* colonies were subcultured every 4 to 6 weeks by transferring about 80 gravid females to a new waxed paper disk with prey using a fine (00000) camel hair brush. The colonies were held in a rearing room at 18L:6D photoperiod, 24° to 28°C, and 75 to 85 percent relative humidity. These rearing procedures are consistent with those used during the selection for carbaryl and permethrin resistances (Hoy and Knop 1981; Roush and Hoy 1981a).

Experimental equipment

Predator tracking was performed with a computerized microprocessor system (J. B. Hoy, Globus, and Norman 1983) (fig. 1). J. B. Hoy and Dahlsten (1984) used the system to analyze the behavior of parasitic Hymenoptera on malathion and protein bait-treated surfaces. The hardware includes an Apple II Plus microcomputer with 48 kilobytes of random access memory and two 133 mm (5¼ inch) floppy disk drives. The computer was interfaced with a Panasonic model WV-1400 video camera through a DS-65 Digisector video digitizer (Microworks, Del Mar, California). One Zenith Datasystems video monitor was used for computer video-output, and another was used to focus and monitor images while tracking was in progress. An Apple Silentype printer printed the actual paths traveled by the mites.

The software for tracking and data analysis was written by P. A. Globus and K. D. Norman, and is commercially available (Computer Sight and Sound, Berkeley, California) (J. B. Hoy, Globus, and Norman 1983). The tracking program, called SPIDER, scans the video image, locates the subject and registers the *x, y* coordinates of the subject at specified intervals. This information is filed on a data-disk. An analysis program calculates the rate of movement in pixels per second. This provides a relative measurement suitable for comparing strains.

Changes were made in the method of calculating rate (J. B. Hoy, Globus, and Norman 1983) to enable point comparisons for both the *x* and *y* coordinates every fifth reading:

```
IF ABS (X4−X) ≤ 2 THEN X1=X
IF ABS (Y4−Y) ≤ 2 THEN Y1=Y
```

This eliminates point accumulation, which would otherwise increase the recorded rate of an inactive specimen. Such artifacts occurred with the original program because the Digisector occasionally registered a one- to three-pixel difference in the *x, y* coordinates between each reading, even when the mites were stationary.

Fig. 1. This computerized tracking system includes an Apple II Plus microcomputer, a video camera, two monitors, two disk drives, and a Silentype printer to establish the activity levels of strains of *M. occidentalis.*

Experimental Technique and Scoring

Strain base-line activity level

The physiological status of females tested was controlled by using unmated females of a known age and hunger level. Larvae were taken from the colonies and placed individually on bean leaf disks (diameter 1.5 cm) that were turned underside up on moist cotton. *Tetranychus urticae* was added as food. Larvae developed into adults in about 3 days at 25° to 28°C. As 0- to 24-hour-old adults, the females were transferred onto fresh bean leaf disks void of all food for 20 to 24 hours at 25° to 28°C.

To measure rate of movement, the starved 1- to 2-day-old unmated females were placed singly on black plastic arenas, 1.1 cm in diameter and 0.2 cm thick. Each arena was placed in a circular depression in a plate made of the same black plastic. Tap water served as a moat. The arenas were cleaned before and after each run using soap and water; care was taken not to contaminate the arenas while setting up new tests. Each female was put on the arena 2 to 4 minutes prior to the test. All mites were tracked at 25° to 28°C and 45 to 55 percent relative humidity. A 34-Watt General Electric cool white circular fluorescent lamp 30 cm in diameter, located 10 cm above the arena, provided uniform lighting for the video camera (luminous intensity = 29 lux [1 footcandle = 10.764 lux]) (J. B. Hoy, Globus, and Norman 1983) (fig. 2). The

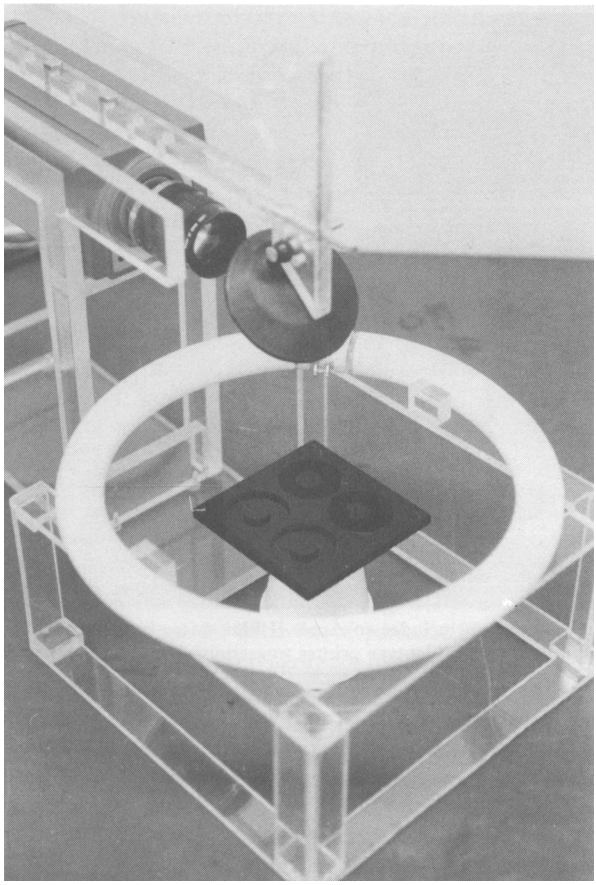

Fig. 2. The video camera is aimed at a mirror, which reflects the image of the arena located at the center and ca. 10 cm below the round, 34-watt cool white fluorescent lamp.

spectrum from the lamp has two peaks: 400 to 475 nm (peak at 440 nm) has 20.2 percent and 550 to 675 nm (peak at 585 nm) has 46.5 percent of the light energy (C. Carlson, Department of Plant and Soil Biology, UC Berkeley, unpublished). Magnified images of the mites were visible on the monitor screen during testing (fig. 3).

To establish AL, the rate of movement of each mite in a test was measured for 30 seconds, three to five consecutive times, with a 1- to 3-minute pause between each run. Equal numbers of mites were tested daily for each strain in a test. Sample size ranged from 15 to 23 individuals per strain. Tracking was conducted between 8:00 and 13:00 each day. Only healthy-looking mites that could walk properly were used for testing, and each mite was tested only once.

Adult males tested were taken directly from the colonies; their age and physiological status (mated or not mated; starved or fed) were unknown. Two criteria affected the selection of males: (1) they had to appear healthy and well fed; and (2) they had to be in motion, and not hovering over a female, when taken out of the colony. Males were tested in the same manner as females.

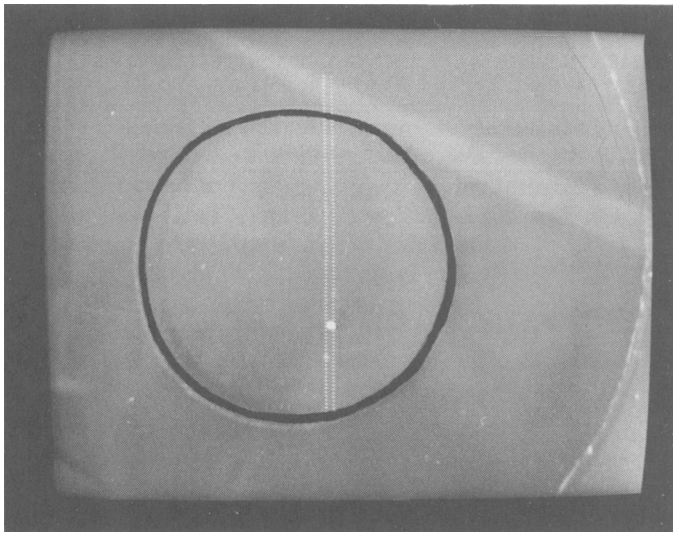

Fig. 3. The computer monitor has a circle drawn on the screen representing the outline of the arena. The female mite, visible as white dot on the screen, was electronically registered by the two vertical white lines that scan the screen searching for and locking in on sharp changes in contrast.

Analysis of variance (ANOVA) and least significant differences (LSD) (Snedecor and Cochran 1980) were used to compare ALs of the strains using a program developed by L. L. Sower (unpublished). A summary of the tests conducted to compare rates of locomotion of the strains is given in table 1. Altogether seven tests were made, five with females and two with males.

Running patterns

The five computer printouts of the tracks run by each of the P-R, COS-R, PF-R, and Wild strain females from test 4 and the P-R and Wild strain males from test 6 (table 1) were assessed to determine whether patterns were detectable, to what extent they occurred, and the relationship between pattern types of these strains and their AL.

Tests with permethrin

A dose-mortality line using permethrin was established for the Wild and P-R strains. Because females often run off permethrin-treated leaf disks into the water-soaked cotton, two lines were calculated for each strain. One included runoff as mortality, and the other excluded runoff. The COS-R strain was used as a susceptible control. The Wild and COS-R strains were treated with permethrin at 1.0 g, 0.5 g, 0.25 g, 0.125 g, and 0.0625 g ai/100 L, and the P-R strain was treated at 16.0 g, 8.0 g, 4.0 g, 2.0 g, and 1.0 g ai/100 L. In total 120 gravid females from each strain were screened at each of

TABLE 1. BASE-LINE ACTIVITY LEVEL (AL) AVERAGES FOR FEMALES OF FIVE STRAINS AND MALES OF TWO STRAINS OF *M. OCCIDENTALIS* (MEASUREMENTS WERE MADE USING A COMPUTERIZED TRACKING SYSTEM AT 25° TO 28°C AND 45 TO 55 PERCENT RELATIVE HUMIDITY)

Test number and strain	Number of mites tested*	Mean AL (S.D.)†	F value‡	Range
		pixels/second	*LSD*	*pixels/second*
Females§				
1. Wild	12	12.1 (3.5)		6.0-16.9
2. Wild	12	12.3 (4.2)	0.65	5.9-19.3
COS-R	12	13.5 (3.1)		8.9-18.6
3. Wild	15	13.5a (3.6)	11.84	7.2-19.9
P-R	15	9.0b (3.7)		3.2-14.8
4. Wild	15	15.7a (3.6)	4.23	9.9-20.8
COS-R	15	14.0a (3.3)	(2.69)	9.0-18.8
P-R	15	11.0b (3.4)		6.4-17.8
PF-R	15	14.2a (4.4)		5.1-21.0
5. COS-R	15	15.8a (2.7)	6.39	11.5-21.5
P-R	15	11.8b (4.0)	(2.23)	5.7-18.2
COP-R	15	13.8ab (3.7)		6.5-19.9
Males¶				
6. Wild	23	14.0a (2.3)	5.90	10.6-18.1
P-R	23	12.3b (2.5)		6.2-18.9
7. Wild	20	15.4a (2.8)	1.16	11.3-21.2
P-R	20	14.5a (3.5)		8.0-19.5

*Five 30-second-long measurements were taken for each female and three for each male tested.

†Means in a test followed by different letters are significantly different. Standard deviation (SD) is given in parentheses.

‡Least significant difference (LSD) for comparisons of more than two means ($P \leq 0.05$) are given in parentheses.

§Females were always 1 to 2 days old, starved approximately 24 hours, and unmated.

¶Males were actively running in the colony when removed for testing.

the five doses. A water control was included. Ten females were put on each disk and scored after 48 hours. Data were analyzed using the POLO program (Robertson, Russell, and Savin 1980). Dose-mortality lines of the data with and without runoff included as mortality were compared by the likelihood ratio test (Savin, Robertson, and Russell 1977).

Genetic analysis of activity level

A genetic analysis of AL was performed using the P-R and Wild strains. Activity levels were simultaneously measured for the parental stock and reciprocal F1 females and males and F2 female progeny ($n = 15$ for each group). For the purpose of these tests, we assumed that the P-R and Wild strains were pure, inbred colonies.

Progenies were produced by mass crosses. Forty to fifty deutonymphal females were isolated from each colony and put on waxed construction paper along with forty to fifty

males and *T. urticae* as food. Three days later, their larval progeny were isolated singly on bean leaf disks. These progeny were held at 25° to 28°C, 18L:6D, and 60 to 80 percent relative humidity. As soon as the progeny had molted to adults, they were starved and their rate of locomotion measured as described previously. Selection and preparation of the males and females for tests were as described previously.

We attempted to repeat the mode of inheritance crosses, but we could not do so. The reciprocal crosses between the Wild and P-R strains demonstrated a one-way mating incompatibility in our second set of tests, yielding significantly fewer eggs and a strongly male-biased sex ratio (Mueller-Beilschmidt 1986).

RESULTS AND DISCUSSION

Strain base-line activity level

Female activity level. The average AL of the Wild *M. occidentalis* females collected directly from the field (table 1, test 1) was significantly lower ($P \leq 0.05$, ANOVA) than the AL recorded for the same strain (table 1, test 4) tested about 4 weeks later. There was also a gradual increase over time in the AL of the P-R and COS-R strains, though their levels of significance were lower than that of the Wild strain: $P \leq 0.095$ (table 1, tests 3 and 5) and $P \leq 0.09$ (ANOVA) (table 1, tests 2 and 5), respectively. Arthropods brought into the laboratory from the field are confronted with sudden changes, so that measurements of fitness (e.g., fecundity or activity) of the first laboratory generation can vary considerably. The fact that all three strains showed a gradual increase in AL over the same time period may mean there is a seasonal influence on their activity.

The Permethrin-resistant strain (P-R) was consistently less active than the Wild strain ($P \leq 0.01$, ANOVA) (table 1, tests 3 and 4). No differences were found between AL averages of the Wild and Carbaryl-OP-Sulfur-resistant (COS-R) strains (table 1, tests 2 and 4). Both the P-R and COS-R are long-established and genetically selected laboratory strains, and yet they have different ALs. We therefore conclude that the low AL of the P-R strain is not necessarily related to the process of laboratory selection or rearing per se. It also agrees with earlier results obtained by Hoy (1977), which indicate that nine generations of inbreeding cause only minimal depression in the fitness of *M. occidentalis.*

In a four-way comparison of the P-R, COS-R, Wild, and PF-R strains (table 1, test 4), the Permethrin-OP-resistant field-recovery (PF-R) strain had an average AL comparable to the Wild and COS-R strains. The fact that the PF-R strain AL is not low, like that of its P-R parental strain, may be due to (1) interbreeding with the native population while in the field or (2) discontinued exposure of the strain to permethrin after recovery from the field. In test 4, as in the other tests, the AL average of the P-R strain was significantly lower ($P \leq 0.05$, ANOVA) than those of the Wild and COS-R strains, which are consistent with the previous tests with these strains.

In test 5 (table 1), the ALs of the P-R, COS-R, and Carbaryl-OP-Permethrin-resistant (COP-R) strains were compared. The AL of the P-R strain was significantly lower ($P \leq 0.01$, ANOVA) than that of the COS-R strain. The average AL (plus or minus the standard deviation) for the COP-R strain was intermediate (13.8 ± 3.7) to the average

ALs of the P-R (11.8 ± 4.0) and COS-R (15.8 ± 2.7) strains (test 5, table 1). The COP-R strain represents a special case in that it is not only a long-established and genetically selected strain, but it has been sequentially and periodically selected with both permethrin and carbaryl in the laboratory for at least 4 years. Although the AL is not statistically different from that of the Wild strain, the intermediate AL recorded for the COP-R strain suggests that the permethrin selections may have reduced the AL of the strain.

The AL averages for the females of the Wild and P-R strains (and their 95 percent confidence intervals) were plotted for all tests (table 1, tests 3 and 4; table 2, tests 1 and 2) that included both strains over a 6-month period (fig. 4). All means of the Wild and P-R strains are significantly different from each other, except for the October 20 measurements. The average rates of locomotion for the P-R strain remained nearly constant over the 7 months. The mean rates of the Wild strain fluctuated slightly more from test to test. Such a difference in variation could be attributable to the Wild strain's being a newly established laboratory colony, and the possibility that it is not as homogeneous as the P-R strain.

Figure 5 presents the highest and lowest rates of the five runs for each of the 15 mites from the four strains in test 4 (table 1). The highest rates are plotted in the order of decreasing AL averages (fig. 5A), and the lowest rates are plotted according to increasing AL averages (fig. 5B). The AL averages for all strains still exhibit the same orders of magnitude of difference; the Wild strain always had higher rates of locomotion than the highest rates recorded for the P-R strain. Conversely, the P-R strain consistently had lower rates than the lowest measurements of the Wild strain. The

TABLE 2. MODE OF INHERITANCE OF ACTIVITY LEVEL OF *M. OCCIDENTALIS* USING THE WILD (W) AND PERMETHRIN-OP-RESISTANT (P-R) STRAINS

Test number and cross	Mean activity level*	Standard deviation	F value	Range
female × male	*pixels/second*			*pixels/second*
1. F1 females; N = 15				
W × W	17.2a	4.6	9.43	7.9-23.7
W × P-R	17.1a	3.7		11.5-24.7
P-R × W	17.7a	3.1		10.3-21.7
P-R × P-R	11.4b	3.5		5.3-18.0
2. F2 females; N = 15				
W × W	15.0a	3.0	2.75	10.5-19.9
(W × P-R) × (W × P-R)	13.0ab	3.8		8.5-20.0
(P-R × W) × (P-R × W)	13.5ab	3.9		4.4-17.9
P-R × P-R	11.3b	3.6		4.7-18.9
3. F1 males; N = 15				
W × W	13.8	2.7	0.52	8.5-18.8
W × P-R	14.5	2.6		9.7-17.9
P-R × W	13.8	2.0		10.9-17.2
P-R × P-R	13.2	3.5		8.0-19.5

*Means within each test that are followed by different letters are significantly different at the $P \leq 0.05$ level according to analysis of variance.

COS-R and PF-R strains' highest rates were similar to those of the Wild strain, and their lower rates were intermediate to those of the Wild and P-R strains. The PF-R strain showed the highest average amount of variation in rates (standard deviation = 4.0).

Male activity level. Comparisons of the ALs of P-R and Wild males are shown in table 1, tests 6 and 7. The results of test 6 indicate the Wild strain had the higher AL average ($P \leq 0.05$, ANOVA). Replications failed to show any differences in AL between the males of the two strains (table 1, test 7, and table 2, test 3). The amount of variation in AL, as indicated by standard deviation, was almost always considerably lower for males than for females, even though the physiological status of the males was not controlled during testing. Also, the difference in AL between P-R and Wild females was highly significant ($P \leq 0.01$) in all but one of seven tests, whereas for males the level of significance was only $P \leq 0.05$ once in three tests. Therefore, we conclude that no differences in AL exist between the P-R and Wild males.

Running patterns of four strains

When starved virgin females and males were put on the smooth black plastic surface of the arena to measure AL, three running patterns could be distinguished (fig. 6):

A. Edge: The mites run in a scalloped pattern along the edge of the arena at least three-quarters of their running time, making frequent stops and occasional turns (e.g., changes of direction) (fig. 6A).

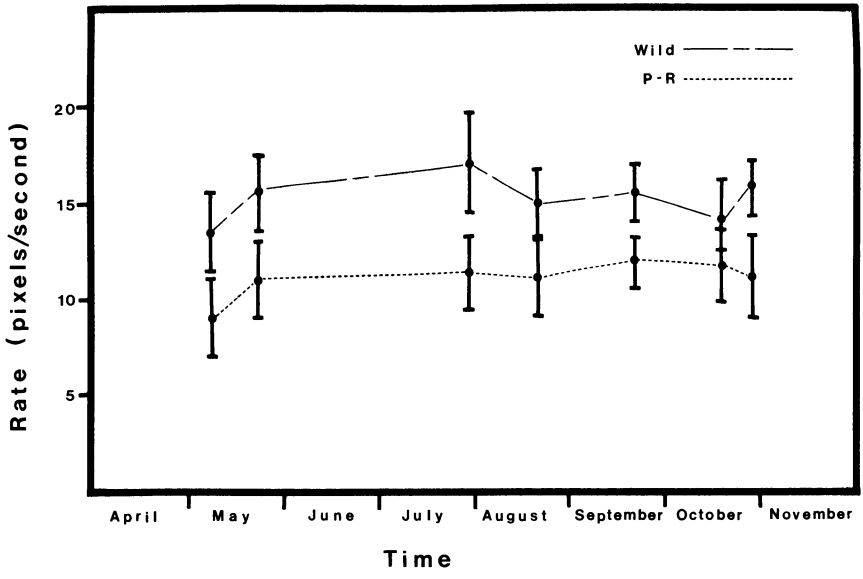

Fig. 4. Average rates of locomotion in pixels per second and their 95 percent confidence intervals for the Wild and Permethrin-resistant (P-R) strains of *M. occidentalis,* obtained in seven independent tests over 6 months.

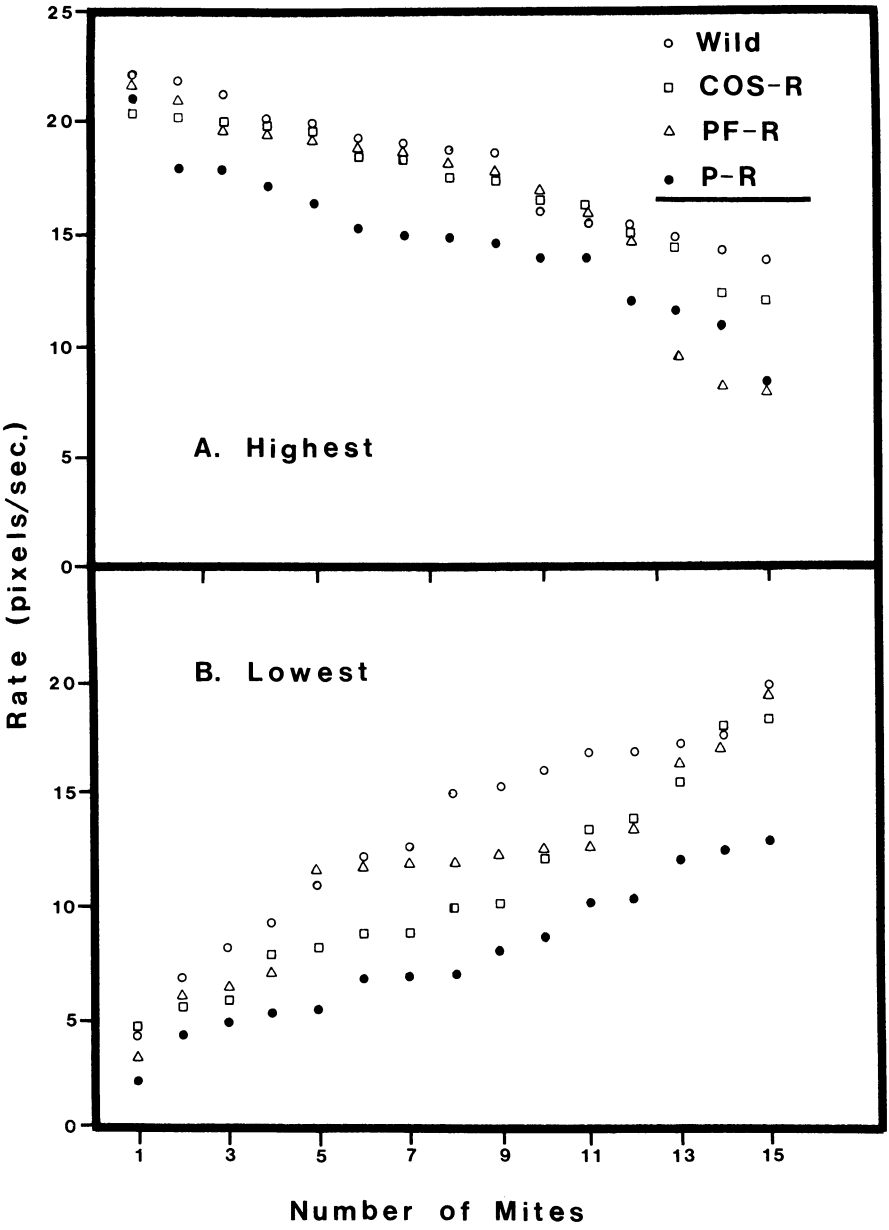

Fig. 5. The highest (*A*) and lowest (*B*) readings of the five consecutive runs made for all females tested from the Wild, P-R, COS-R, and PF-R strains placed in order of decreasing (*A*) or increasing (*B*) rates.

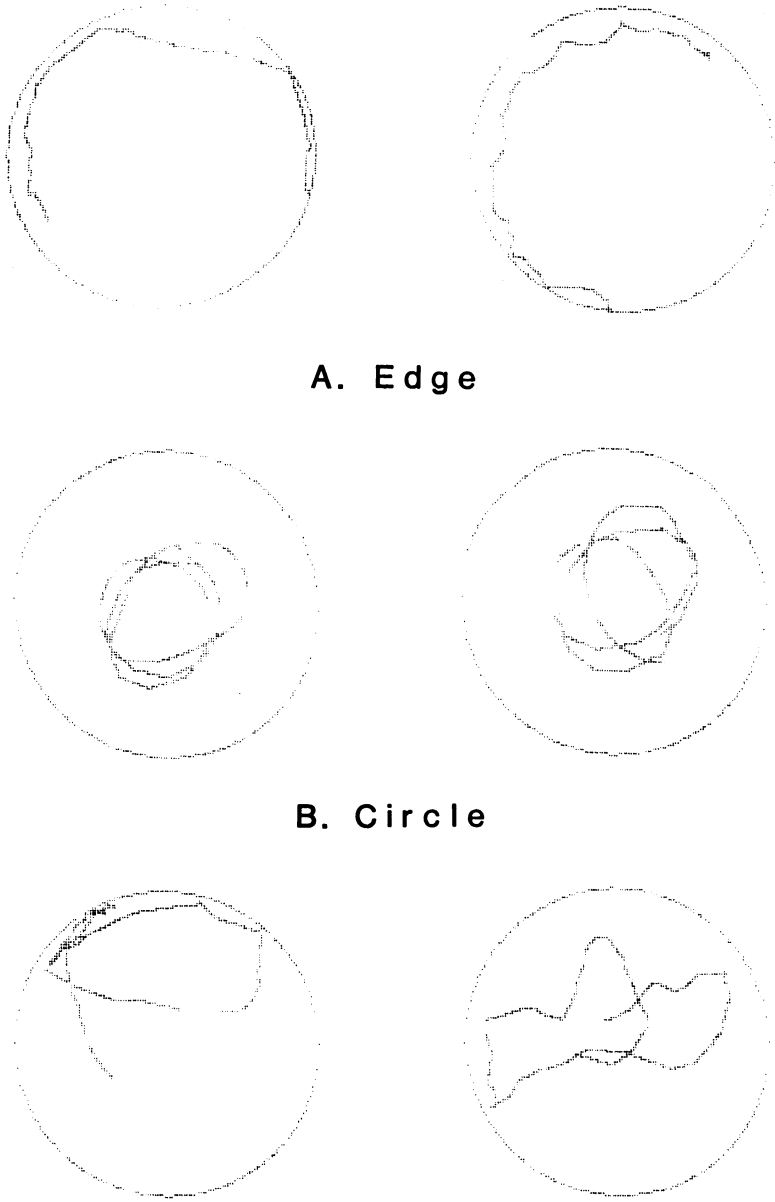

Fig. 6. The three different running patterns displayed by the starved virgin (1- to 2-day-old) females of *M. occidentalis*: (*A*) edge, (*B*) circle, and (*C*) nonspecific.

B. Circle: The mites run in circles, usually without making stops or changing direction, and only seldom encounter the edge of the arena (fig. 6B).

C. Nonspecific: The mites run in all directions, making occasional turns and stops. This pattern includes all track patterns not belonging to types A or B (fig. 6C).

The five computer printouts of the track patterns obtained for each P-R, COS-R, PF-R, and Wild strain female from test 4 (table 1) and the P-R and Wild strain males from test 6 (table 1) were assigned to one of the three categories based on the criteria given above; their proportions were compared by chi-square analysis. Of the 15 females measured for each strain (test 4) the numbers that exhibited one track pattern exclusively in at least four of their five measurements are given in table 3. The Wild strain, which had the highest AL, had the highest number of exclusive circle runners (9 of 15). The P-R strain, the slowest strain, had the most mites running only the edge pattern (7 of 15). The COS-R and PF-R strains had almost equal proportions of mites in the circle and edge categories. The nonspecific pattern was least frequently observed in all four strains. The possible significance of these observations will be discussed later.

The males (table 1, test 6) did not run in circles; they displayed only the nonspecific- and edge-running patterns. Nor did males exclusively run one pattern or the other. Therefore, the total number of runs of all males tested in each strain are given only for the two remaining categories (table 3). The majority of Wild males ran in a nonspecific pattern, whereas males of the P-R strain primarily ran along the edge. It appears that both the activity level and the pattern of activity differ between the males and females of the P-R and Wild strains.

Tests with permethrin

Large numbers of females ran off the leaf disks after treatment with permethrin, especially at the lower doses tested relative to the tolerance of each strain (table 4). There appears to be a relationship between the dose response of a strain and the amount of runoff. Both the Wild and COS-R strains had unusually high amounts of runoff at the three lowest doses (0.0625 g, 0.125 g, and 0.25 g ai/100 L water). At 0.5 g ai/100 L water, the amount of runoff dropped significantly ($P \leq 0.05$, ANOVA) for these two colonies. Maximum runoff by both strains occurred at 0.125 g ai/100 L water for both strains. An inflection of the survival values for the Wild and COS-R strains corresponds to the point of maximum runoff (table 4). Maximum runoff for the P-R strain occurred at the two lowest doses tested (1.0 and 2.0 g ai/100 L water).

Dose-mortality curves were calculated in two ways: (1) with runoff included as mortality, and (2) with runoff excluded. The slopes of the curves for the P-R and COS-R strains calculated with and without runoff included as mortality were significantly different (chi-square = 11.86 and 7.65, respectively), whereas they were not different for the Wild strain. The LD_{50} values of the lines, including and excluding runoff, were significantly different for all three strains (Wild, chi-square = 9.92; P-R, chi-square = 14.08; COS-R, chi-square = 17.99).

Permethrin is known to have a repellent effect on arthropods (Grafton-Cardwell and Hoy 1985; Thomson 1982), which might account for the runoff associated with permethrin treatments. The dose-mortality tests show that the runoff caused by permethrin treatments makes a significant shift in the slope and LD_{50} values of the strains tested. Runoff individuals might represent a gene pool that was inadvertently,

and consistently, selected out of the P-R population during selection with permethrin. A low AL may be one of the measurable consequences of the unintentional selection.

Genetic analysis of activity level

Activity level averages of the reciprocal F1 (P-R × W and W × P-R) females did not differ from that of the Wild strain control in the initial tests. The reciprocal F1 females

TABLE 3. TRACK PATTERNS OF INDIVIDUAL MALES AND FEMALES OF FOUR STRAINS OF *M. OCCIDENTALIS* DURING ACTIVITY LEVEL TESTS, CATEGORIZED ACCORDING TO THEIR RUNNING TYPE

*Females**		Number of mites running at least 4 of 5 times exclusively in one running category† (with variance‡)		
Strain tested	Average rate (with SD)	Circle§	Edge¶	Nonspecific#
	pixels/second			
Wild	15.7	9	3	1
	(3.6)	(1.90)	(1.55)	(0.92)
P-R	11.0	2	7	2
	(3.4)	(1.30)	(1.93)	(1.30)
COS-R	14.0	5	4	2
	(3.3)	(1.82)	(1.70)	(1.30)
PF-R	14.2	5	3	0
	(4.3)	(1.82)	(1.55)	
*Males**		Total number of runs in each category† (with variance‡)		
Strain tested	Average rate (with SD)	Circle§	Edge¶	Nonspecific#
	pixels/second			
Wild	14.0	0	30	39
	(2.3)		(4.12)	(4.11)
P-R	12.3	0	47	22
	(2.5)		(3.90)	(3.90)
Wild	15.4	0	22	38
	(2.8)		(3.74)	(3.74)
P-R	14.5	0	35	25
	(2.5)		(3.82)	(3.82)

*For the tests with females, N = 15 for each strain, with 5 runs made per female. For the males, N = 23 in the first two tests and 20 in the second two tests, with 3 runs made per male.

†The number of mites in each row does not necessarily correspond to the total number tested for that strain, because not every mite running pattern could be included in one of the categories.

‡Variance (given in parentheses) is calculated as $\sqrt{np(1-p)}$ for each number, where n = the number in the sample and p = the sum of all samples. There is no variance if value = 0.

§Distinguished as running usually in one direction, without stops and without encountering the edge of the arena.

¶Running in a scalloped manner along the edge of the arena, with many stops and occasional changes of direction.

#Characterized by frequent stops, turns, and complete changes of direction; includes all runs that are neither circle nor edge type.

TABLE 4. COMPARISON OF THE AVERAGE AMOUNTS OF RUNOFF AND SURVIVAL OF FEMALES OF THREE *M. OCCIDENTALIS* STRAINS AFTER TREATMENT WITH DIFFERENT RATES OF PERMETHRIN

Strain tested	LD_{50} (95% C.L.)* With runoff	LD_{50} (95% C.L.)* Without runoff	Dose†	Mean percent Survival	Mean percent Runoff‡
Wild			0.0625	39	29a
	0.12	0.05	0.125	22	42a
	(0.08-0.17)	(0.02-0.09)	0.25	26	33a
			0.5	19	8b
			1.0	2	0b
			water	83	3b
COS-R			0.0625	41	32a
	0.13	0.02	0.125	18	43a
	(0.06-0.19)	(0.001-0.07)	0.25	16	27a
			0.5	28	12b
			1.0	4	3b
			water	82	0b
P-R			1.0	73	23a
	4.08	3.32	2.0	59	12ab
	(3.52-4.68)	(2.49-4.23)	4.0	47	4b
			8.0	29	5b
			16.0	0	1b
			water	93	6b

*120 gravid adult females were tested with each dose of permethrin and with water as a control over 4 days. Slopes with and without runoff for the Wild strain were 1.54 (±0.20) and 1.07 (±0.17), for the COS-R strain 1.42 (±0.2) and 0.75 (±0.16), and for the P-R strain 1.81 (±0.2) and 2.78 (±0.22), respectively.

†g ai/100 L.

‡Means for each strain followed by different letters are significantly different at $P \leq 0.05$ according to analysis of variance.

and the Wild parent control had AL averages significantly higher ($P \leq 0.01$, ANOVA) than the AL average of the P-R parent strain (table 2, test 1). The reciprocal F2 female progeny had average ALs intermediate to those of the Wild and P-R controls (test 2). The AL averages for the Wild and P-R controls were significantly different in both the F1 ($P \leq 0.01$, ANOVA) and F2 ($P \leq 0.05$, ANOVA) tests (tests 1 and 2), as expected. The results are compatible with a model in which the trait determining higher AL (Wild strain) is dominant over that for lower AL (P-R strain). A mating incompatibility between one of the reciprocal crosses made it impossible to replicate the tests. Why the mating incompatibility occurred in the second test, but not in the first, is unknown.

Other researchers have determined that the mode of inheritance for locomotor activity is influenced by single major genes. Choo (1975) found that the walking behavior of *Drosophila melanogaster* (Meigen) seemed to be controlled by a major gene, with the slow walking response dominant. Van Dijken and Scharloo (1979a, b) found that in *D. melanogaster* the largest effect on locomotion is associated with the X-chromosomes, and suggested that the trait might be narrowed to one or a few loci. Most genetically analyzed behaviors do not fit the single-gene model, however. Instead,

they appear to be polygenically determined (Hirsch and McGuire 1982). Though mode of inheritance is still unclear, these tests indicate that the AL in *M. occidentalis* females is a heritable trait and that the differences are consistent.

The F1 male progeny and males of Wild and P-R controls showed no differences in AL averages (table 2, test 3). Males differ from females not only in running activity and patterns, but also in the inheritance of AL. These differences might be related to the unusual genetic system of *M. occidentalis* mites, referred to as parahaploidy (Hoy 1979; Nelson-Rees, Hoy, and Roush 1980) or pseudo-arrhenotoky (Schulten 1985). In this system all eggs are fertilized. During embryogenesis the paternal chomosomes heterochromatize in those eggs destined to become males, making them functionally haploid (Nelson-Rees, Hoy, and Roush 1980).

Significance of AL as a measure of quality

The computerized tracking system employed in this study to record the rates and patterns of male and female *M. occidentalis* locomotion did indeed demonstrate repeatable differences for both rate and pattern of locomotion between females of the strains. The computer-video system is an easy method for tracking and quantifying the movement of small nonflying arthropods on a two-dimensional scale (J. B. Hoy and Dahlsten 1984; J. B. Hoy, Globus, and Norman 1983). This system is less laborious and definitely more accurate than methods previously described, such as the use of dissecting microscopes to hand-trace an organism's path, later analyzing the path by means of a curvimeter (Everson 1980; Hoy and Smilanick 1981), or the implementation of magnified grids of an ocular micrometer (Fransz 1974; Sabelis 1981), or the employment of long-exposure infrared photography in a light-tight box (Penman and Chapman 1980).

Essentially, locomotion can be broken down into two components: velocity (distance covered per unit time) and continuity (frequency and duration of locomotor activity). Sabelis (1981) measured these two components separately. He defined the walking activity of phytoseiids as "the fraction of the observation time the animal spends walking," referred to here as continuity. In this study, quantification of activity level for five strains of *M. occidentalis* was based on the continuity and velocity of their locomotion under laboratory conditions.

Physiological status does affect the locomotor activity of *M. occidentalis* males and females. Standardization of their level of hunger, mating status, and contacts with other mites is necessary. In preliminary tests, some adult females taken randomly from the colony sat still for an extended period of time when put on the test arena, especially those with a plump and gravid appearance. Slender-looking females would usually take off running immediately. Adult males are typically more active than females. In the laboratory, males run continuously through the colony, and they become stationary only when hovering over a female deutonymph, during copulation, or when feeding. We observed that males isolated as immatures and subsequently starved for 24 hours as adults were almost motionless when placed upon the plastic test arena, a contrast to the behavior of the females treated similiarly. For that reason, only actively running adult males taken directly from the colony were used for testing. Other factors affecting locomotor activity include handling disturbances prior to testing (Sabelis 1985) and the use of plastic test arenas (Everson 1980); these were accounted for by standardizing

the histories of the mites. Nevertheless, these AL measurements are relative, and only provide comparative values under the specified laboratory conditions.

The effects of hunger or starvation on the locomotor activity of female phytoseiids has been considered by Fransz (1974) and Sabelis (1981). They found that hunger had little effect on the fraction of time spent walking by *M. occidentalis,* but Sabelis (1981) did indicate that hunger caused a moderate increase in velocity (speed). According to Fransz (1974), two or more days of food deprivation, which induces a state of starvation as opposed to hunger, can instigate changes in the activity and search behavior of *M. occidentalis.*

The physiological basis for the differences in ALs and running patterns between these strains might represent differences in the intensity of a response, and not a qualitative behavioral difference. Such a strain by treatment effect could also lead to differences among strains in their sensitivity to hunger or light, and consequently to differences in running patterns and ALs.

Analysis of the running patterns displayed by the strains of *M. occidentalis* suggests a relationship between their track patterns and ALs. The three categories of running (edge, circle, and nonspecific) constitute different frequencies of turning and stopping, both of which decrease rate of locomotion. The P-R strain, which had the lowest ALs, displayed mostly edge running; the edge behavior is characterized by frequent stops and occasional turns (e.g., changes of direction). Sabelis (1981) and Sabelis and Dicke (1985) have described and discussed such an "edge-oriented walk" by phytoseiids, and suggest that it increases the efficiency of the predator's search for spider mite colonies on a leaf. The Wild strain, which had the highest ALs, ran predominantly in circles. Circle running typically involves little or no stopping or changing of direction. The nonspecific running resembles the patterns observed by Hoy and Smilanick (1981) for *M. occidentalis* females on bean leaf disks with silk and kairomone residues of *T. pacificus* McGregor. These pattern differences appear to account, at least partially, for the differences in rate of locomotion between the strains as measured in mean pixels per second.

The circle-running behavior characteristic of the Wild strain females is especially noteworthy and difficult to interpret. There does not seem to be any obvious motive that would elicit such a running pattern. A possible external factor that could have instigated such a response was the bright round fluorescent light over the arena. Even though *M. occidentalis* mites do not have compound eyes or ocelli, they are sensitive to light and can measure time; short photoperiods induce diapause in females (Hoy 1975a, b). To our knowledge, the phototactic behavior of *M. occidentalis* has never been studied, but females are likely to be negatively phototropic, based on their general preference for the underside of the leaves and for shadier areas on their host plant (Chant and Fleschner 1960; Field and Hoy 1985; Kinn and Doutt 1972).

Starvation could have had an effect on running behavior of *M. occidentalis* females. After a 24-hour period of starvation, the majority of the Wild strain females, and to a lesser extent the COS-R and PF-R females, became flat and translucent. In contrast, the P-R females usually maintained an opaque color and a plumper appearance. The degree of starvation, which could be based on differences in metabolic rates of the strains, could elicit different running patterns. As mentioned earlier, hunger is known to affect phytoseiid behavior. Fransz (1974) postulated that searching behavior, and therefore activity, may be hunger dependent. Field and Hoy (1985) found that well-fed diapaus-

ing females of *M. occidentalis* were more likely to seek shelter under black crepe paper bands in a bean plant system than hungry diapausing females. Similarly, they found that starved females more readily exhibited aerial dispersal behavior than well-fed females.

The circle-running behavior exhibited by the Wild strain might be due to a combined effect of their more advanced stage of hunger and the round fluorescent light used for testing. Assuming that *M. occidentalis* females do possess light-sensitive cells, the amount of light these cells receive could increase as emaciation of the females increases. As a consequence of starvation, such light-sensitive cells could evoke altered behavioral responses to light. We speculate, consequently, that under these conditions a female mite that continually attempts to turn away from a circular light source would end up running in circles.

The possiblity that female mites are sensitive to a humidity gradient established by the water surrounding the 1.1 cm diameter circular arena used in these tests, and that such a sensitivity results in the circle-running pattern seen in the Wild strain, is less likely. Penman and Chapman (1980) were unable to detect significant variations in locomotor activity (mm/minute) of *M. occidentalis* at different relative humidities. Mori and Chant (1966) found that *Phytoseiulus persimilis* individuals did not discriminate between various relative humidities either, though they were observed to be more active under lower relative humidities ($\leq$ 76 percent), whereby activity was based on the presence or absence of movement. Temperatures in the range of 15° to 30°C also exerted little influence on the speed or continuity of locomotion in different phytoseiid species (Penman and Chapman 1980; Sabelis 1981).

The relationship of the differences between the rates and patterns of locomotion that were observed for the strains in the laboratory and the same strains in the field has not been established by this work. A better understanding of the influences of external factors (e.g., humidity, light, and availability of prey) on behavior of *M. occidentalis* is essential for understanding the impact of laboratory maintenance and its effect on this predator's performance when released into the field. Our work did show that (1) AL, as defined in this paper, can be measured easily, and (2) consistent and repeatable differences occur between laboratory strains under the specified laboratory conditions.

Ideally, AL measurements would only be one component of a quality control program. Other techniques might include gel electrophoresis to survey variations in enzymatic and nonenzymatic proteins, tests for changes in fecundity, sex ratio, and longevity, and field tests of key behaviors, such as diapause and aerial dispersal (Chambers 1977). Activity level measurements using the computerized tracking method do provide a repeatable and quantitative measure of this predator's walking behavior under controlled conditions, and the measure could be used to evaluate the activity levels or patterns of other small arthropods in a two-dimensional test system.

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