Biology of *Harmonia axyridis* (Coleoptera: Coccinellidae) in Winter Aggregations

C. A. NALEPA, K. A. KIDD, AND K. R. AHLSTROM

Biological Control Laboratory, Plant Protection Section, North Carolina Department of Agriculture, P.O. Box 27647, Raleigh, NC 27611

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ABSTRACT Adults of the introduced coccinellid *Harmonia axyridis* (Pallas) were collected at aggregation sites in the autumn of 1993 and 1994 and described in terms of sex, size, and phenotype. Mating status and ovariole numbers of females and parasitism rates of both sexes were obtained by dissection. Females were larger than males, and beetles of both sexes from high elevations were larger and darker than their counterparts from lower elevations. In 1993, 11.8% of females had sperm in their spermathecae but 41.4% of females were mated in 1994. Adults were parasitized by *Strongygaster triangulifera* (Loew) (Tachinidae) at rates of 14.2 and 1.4% in 1993 and 1994, respectively.

KEY WORDS lady beetle, aggregation, parasitism, sex ratio

LARGE NUMBERS OF THE multicolored Asian lady beetle *Harmonia axyridis* (Pallas) originating from Japan, the Republic of Korea, and the former USSR were released in the United States during the late 1970s and early 1980s (L. R. Ertle, personal communication). Their 1st reported recovery was in Louisiana in 1988 (Chapin and Brou 1991); however, whether intentional releases or accidental introductions at seaports resulted in establishment is controversial (Day et al. 1994, Tedders and Schaefer 1994). Regardless of the mode of entry, *H. axyridis* has since spread rapidly in the eastern half of the country, and currently ranges from Florida to Québec and west to Texas and Missouri. It is also reported from Washington, Oregon, and British Columbia, Canada (P. W. Schaefer, personal communication). During the spring and summer, these lady beetles are effective predators of a wide variety of aphids, as well as coccids and other insects (Tedders and Schaefer 1994). In the autumn they migrate from feeding habitats to hibernation sites, where they pass the winter in aggregations (Tanigashi 1976, in Obata 1986). In the United States the overwintering sites are often houses or other structures, where they can be a nuisance (Kidd et al. 1995).

Because *H. axyridis* is newly established in the United States, little is known of its biology in this country. The ease with which these coccinellids may be collected when they begin forming aggregations in autumn allowed us to sample and study adults from one site in Virginia and from a variety of locations in North Carolina.

Materials and Methods

Beetles were collected shortly after they arrived at their aggregation sites in the autumn of 1993 and 1994 from buildings in both urban and agricultural areas. In 1993, samples were taken from 10 locations: 9 in North Carolina and 1 in Virginia. Five sites in 3 counties were designated high elevation sites: 2 sites in Alleghany County, North Carolina, 2 sites in Watauga County, North Carolina (≥915m), and 1 site in Highland County, Virginia (550 m). Lower elevation sites (<366 m) were all in North Carolina: 2 sites in Wake County, and 1 site each in Burke, Rutherford, and Vance counties. Beetles were collected at higher elevation sites in North Carolina from 26 to 28 October 1993. The beetles from Virginia were collected on 28 October 1993 by Paul W. Schaefer (USDA Beneficial Insects Laboratory, Newark DE) and shipped to the authors on 2 November 1993. Samples were taken between 28 October and 17 November 1993 at the lower elevation sites. In 1994, beetles were collected between 19 October and 9 November from 14 locations in 10 counties in the western half of North Carolina. These collection sites were widely distributed over the area, with 4 counties designated as high elevation (Alleghany, Watauga, Jackson, Buncombe), and 6 as lower elevation (Davidson, Montgomery, Wake, Burke, Vance, Rockingham). Five counties were sampled in both 1993 and 1994, but just 4 sites were sampled in both years.

All insects were transported from the collection sites in insulated containers containing artificial ice packs, then refrigerated at ≈ 9°C until analyzed. The length of the insects was measured from the anterior edge of the pronotum to the posterior edge of the elytra (1993 sample), and the insects were sexed using the shape of the 5th abdominal sternite (1993 and 1994 samples).

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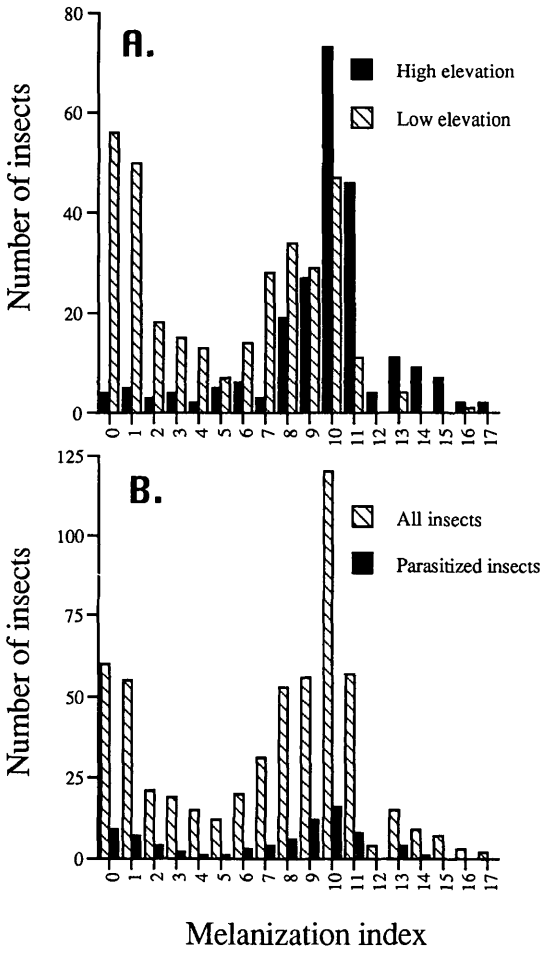

Fig. 1 (A) Degree of melanization of adult *H. axyridis* at high elevation (≥550 m) and lower elevation (<366 m) sites. On the x-axis, numbers between zero and 10 represent a progressive increase in the number of spots on the right elytron; numbers between 11 and 17 represent increasing numbers of fusions in the 10-spot type (see text). (B) Phenotypes of *H. axyridis* parasitized by *S. triangulifera* compared with all phenotypes sampled. Spot number as in (A).

All *H. axyridis* collected in the eastern United States to date are the nonmelanic form (i.e., elytral color varies from yellow to brick red) with 1 to 10 melanic spots on each elytron. Varying degrees of spot fusion are also found (Chapin and Brou 1991, Tedders and Schaefer 1994, Kidd et al. 1995). We characterized phenotypes of adults collected in 1993 by counting the number of spots on the right elytron, and assigning each beetle to a number category. Numbers between zero and 10 represent a progressive increase in the number of spots; numbers greater than 10 represent an increasing number of spot fusions of the 10-spot type. Numbers on this scale, then, are a rough indication of the degree of melanization of the phenotypes. Spots varied in size and shape, and insects assigned to a certain spot number category did not necessarily have the same spot location pattern. The 3-spot type, for example, had 6 pattern permutations.

Table 1. Mean ± SD body lengths (mm) of adult *H. axyridis* collected in 1993

	No.	Body length
Male	283	6.04 ± 0.37
Female	277	6.51 ± 0.41
High elevation adults	233	6.44 ± 0.42
Low elevation adults	327	6.16 ± 0.44

Beetles were dissected and examined for parasitoids in both 1993 and 1994. In 1993, larvae present in the body cavity were preserved in 80% ethanol and later measured. Adult *H. axyridis* from 2 sites, Vance County, North Carolina, and Highland County, Virginia, were held at room temperature and monitored for the emergence of parasitoids.

The mating status of females was determined by removing the chitinous spermatheca (illustrated in Chapin and Brou 1991), crushing it on a slide, and examining it microscopically under phase contrast illumination (n = 246 in 1993, n = 374 in 1994). In 1993, lady beetles were held as a group in either plastic bags or paper cartons under refrigeration until dissection. It was assumed that no mating would take place under these conditions. Lady beetles collected in 1994 were placed individually into gelatin capsules on the day they were collected and later dissected to determine mating status. In 1993, ovaries of females from Burke and Wake counties, North Carolina, and Highland County, Virginia, were removed and preserved; the ovarioles in one ovary (not consistently right or left) were later counted (n = 97).

Data were analyzed using chi-square, and by regression and analysis of variance (ANOVA) using SYSTAT (1992). Vouchers of *H. axyridis* and its associated parasitoid *Strongygaster triangulifera* (Loew) (Tachinidae) have been deposited in the North Carolina Department of Agriculture Insect Collection.

Results

The phenotypes of adults collected from higher elevation sites were dominated by the heavily spotted types, whereas adults from lower elevation sites exhibited a bimodal distribution in their degree of melanism (Fig. 1). The mean ± SD body length of adults was 6.27 ± 0.45 mm (n = 560). Females were significantly larger than males, and adults from high elevations were significantly larger than adults from lower elevations (Table 1). There was, however, no statistical interaction between sex and location (Table 2). The overall sex ratio of *H. axyridis* from the 1993 collections was 1:1 (275 females, 282 males), and varied from 0.7:1 to 1.4:1 among sites. Twenty-nine of 246 dissected females (11.8%) had sperm in their spermathecae; the range was from no mated females at one site in Wake County, North Carolina, to 25% mated fe-

Table 2. ANOVA factors affecting body size

Source	SS	df	MS	*F*	*P*
Sex	32.09	1	32.09	247.39	<0.001
Elevation	12.12	1	12.12	93.41	<0.001
Sex*Elevation	0.21	1	0.21	1.58	0.21
Error	72.13	556	0.13		

males in the collection from Highland County, Virginia (Table 3). In 1994, females significantly outnumbered males by 1.2:1 (461 females, 370 males) ($\chi^2 = 9.96$, $P < .005$), ranging from 0.3:1 to 3.6:1 among sites. There was a higher proportion of mated females in 1994 than in the previous year: 41.4%, ranging from none in a Burke County site to 69.6% in a sample from Wake County.

Ovaries were undeveloped, consistent with published data on dormant coccinellid adults (Hagen 1962, Hodek and Landa 1971). The number of ovarioles per ovary ranged from 22 to 42, a wider range than the 27 to 39 reported by Dobzhansky (1924). The mean and mode number of ovarioles per ovary was 31 ($n = 97$) (Fig. 2). The number of ovarioles per ovary was statistically unrelated to female body length.

In 1993, dipteran parasitoid larvae were present in coccinellids from all sampled sites; rates of parasitism ranged from 3.0 to 31.1% (Table 3). Overall, 79 of 557 dissected adults were parasitized (14.2%); 35 of these beetles were male (44.3%) and 44 were female (55.7%). These differences are not significant (chi-square). In 72 of the 79 cases of parasitism a single larva was present, but 6 beetles contained 2 larvae, and 1 beetle had 3 larvae. In 1994, parasitoids were recovered from just 7 of 14 sampled sites. Rates of parasitism ranged from 0.0 to 6.5%, with an overall parasitism rate of 1.4% (12 of 831 dissected adults); no elevational patterns were evident. One hymenopteran larva was present in a beetle from Buncombe County.

Adult dipterans reared from coccinellids held at room temperature were identified (by K.R.A.) as *S. triangulifera* (3 males and 11 females). Measurements of the tachinid larvae allowed only rough approximation of their developmental stage. About half of them were estimated to be 2nd instars and half 3rd instars. A few probable 1st instars were noted. Observations during laboratory rearing support Thompson's (1954) suggestion that the larva issues from the host to pupate. Parasitoid larvae were observed crawling on the substrate of rearing chambers and pupae were collected from the excelsior provided the beetles for shelter. The tachinid did not prefer specific phenotypes of the beetle; the distribution of phenotypes of parasitized beetles is similar to the distribution for the entire sampled population (Fig. 1).

Discussion

Like other coccinellids (Smith 1966), adult *H. axyridis* are sexually dimorphic in body size. Elevational differences in body size and degree of melanism occurred in both sexes; *H. axyridis* adults collected from high elevations were larger and more melanized than those from lower elevations. Polymorphism in this species is based on a series of multiple alleles and is known to vary with prey, season, and geographic location (Dobzhansky 1933, Komai 956); nonrandom mating also plays a role (Osawa and Nishida 1992). Temperatures experienced during the prepupal and early pupal stages are particularly influential in determining the phenotype of the adult (reviewed by Hodek 1973). Low temperatures prolong pupal development, which results in more and larger spots; acceleration of development leads to reduction of spotting (Tan and Li 1932). In this study, the difference in phenotypes between collections from the Piedmont and the mountains suggests that *H. axyridis* may not travel long distances between feeding/pupation sites and aggregation sites.

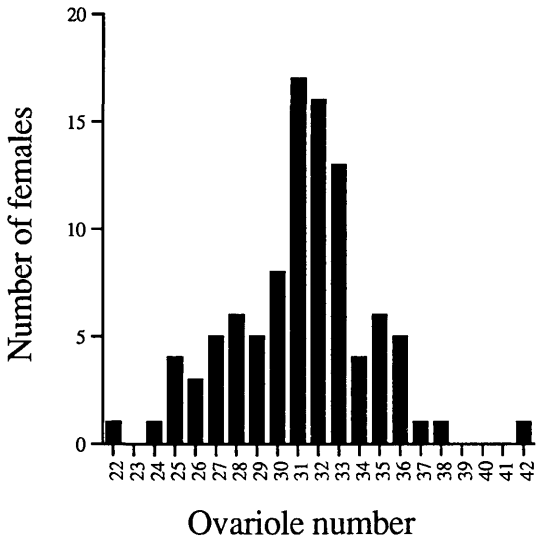

Fig. 2. Ovarioles per ovary in dissected *H. axyridis*.

Table 3. Percentage of parasitism and females mated in *H. axyridis* in 1993 ($n = 557$)

Site	County	No. dissected 1993/1994	% females mated 1993/1994	% parasitism[a] 1993/1994
1	Highlands, VA	100	25.0	13.0
2	Alleghany, NC	32/101	14.3/40.0	9.4/3.0
3	Alleghany, NC	33	11.8	20.6
4	Watauga, NC	26	7.7	11.5
5	Watauga, NC	40	8.7	7.7
6	Burke, NC	100/89	11.8/0.0	3.0/1.1
7	Vance, NC	98/31	7.8/62.5	15.3/6.5
8	Wake, NC	45	10.0	31.1
9	Wake, NC	41/59	0.0/69.6	19.5/1.7
10	Rutherford, NC	42	–	23.8

Sites 1–5 are at high elevations (≥550 m); sites 6–10 are at lower elevations (<366 m).

[a] Parasitized by *S. triangulifera* (Tachinidae).

The significantly higher proportion of females in the 1994 collection is unexplained; many factors influence sex ratio. In this study, however, beetles were collected ≈ 1 wk earlier in 1994 than in 1993. A temporal variation in the arrival of the sexes at aggregation sites may account for the difference.

The fact that some females of *H. axyridis* had mated before arriving at their winter aggregation is not unusual. Although diapausing coccinellids usually mate at the end of hibernation, before aggregations disperse, some mating may occur before diapause (Hodek 1973). No sperm, for example, were found in winter collections of *Semiadalia undecimnotata* Schneid (Hodek and Landa 1971); in a different population of the same species, however, 9% of the females were mated (unpublished data of Iperti 1970 in Hodek and Landa 1971). Variation in the number of mated *H. axyridis* females in this study may be the result of climatic differences; earlier emergence of the final generation of the year in some locales may result in the presence of receptive females before aggregation. Because *H. axyridis* can be long lived (up to 3 yr, Savoïskaya 1970), it is also possible that some mated females in our study were from a generation previous to the one maturing late in the year. In Japan, there are 2 generations per year and it is in the 2nd generation of adults that mating does not occur until the following spring (Sakurai et al. 1992). Male *H. axyridis* will copulate with a female regardless of her mating status (Obata 1988); therefore, whether or not they were virgins when they entered the aggregation in fall, all females emerging from aggregations in early spring are likely to mate.

The parasitoid *S. triangulifera* is widely distributed in the Nearctic: from British Columbia to Nova Scotia in Canada, and south to California, Mexico, and Georgia (Stone et al. 1965). It has hosts in many beetle families, and there are also records of parasitism on Lepidoptera, Dermaptera, and Hemiptera (Thompson 1954, Sabrosky and Braun 1970). We found just 1 record of *S. triangulifera* parasitism rates; the coccinellids *Coleomegilla maculata lengi* Timberlake and *Coccinella trifasciata perplexa* Mulsant were parasitized at very low levels (<1%) in Ontario (Smith 1960).

The *H. axyridis* used in our study were collected shortly after they had arrived at aggregation sites and then refrigerated until dissection. The presence of predominately older parasitoid larvae suggests that beetles were parasitized at their feeding sites before intiating aggregation; *S. triangulifera* then overwinters in the body of its host.

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