MORPHOMETRIC STUDIES IN *NEODRYINUS TYPHLOCYBAE* (ASHMEAD) (HYMENOPTERA: DRYINIDAE) DIAPAUSING LARVAE AND COCOONS IN ITALY: A MULTIVARIATE APPROACH[1]

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ABSTRACT: Univariate and multivariate one-factor analyses of variance (ANOVA and MANOVA) were applied to examine the effect of the "gender" factor on some measurements of diapausing larva and cocoon in *Neodryinus typhlocybae* (Ashmead) (Hymenoptera Dryinidae). This species was introduced into Italy in 1987 for the biological control of the Nearctic planthopper *Metcalfa pruinosa* (Say) (Hemiptera Flatidae). Five measurements were taken of each specimen: length (Le) and width (Wi) of the cocoon wall; length (le) and width (wi) of the larval chamber and length of the diapausing larva (dll). Results of MANOVA indicate that all the considered morphometric variables are influenced by the "gender" factor. Results of ANOVAs indicate that length and width of the larval chamber and the length of the diapausing larva are the variables more strictly correlated with the gender.

KEY WORDS: *Neodryinus typhlocybae,* Hymenoptera, Drynidae, planthopper parasitoids, *Metcalfa pruinosa,* Hemiptera, Flatidae, morphometric traits, sexual dimorphism, multivariate, MANOVA, *Pittosporum tobira,* Pittosporaceae

In 1987, *Neodryinus typhlocybae* (Ashmead) (Hymenoptera: Dryinidae) was introduced to the Veneto Region of Italy for the biological control of the Nearctic planthopper *Metcalfa pruinosa* (Say) (Hemiptera: Flatidae) (Girolami and Camporese, 1994). *Neodryinus typhlocybae* is a North American parasitoid of flatid planthopper nymphs with an arrhenotokous parthenogenesis and thelytokous sexual reproduction (Olmi, 1999). Over the past ten years, this insect has been successfully released in many urban and agricultural areas of Italy, France, Switzerland, Slovenia and Croatia (Lucchi and Wilson, 2003; Alma et al., 2005).

The life cycle of *N. typhlocybae* is characterized by overwintering of the diapausing larva inside a silky cocoon and adult emergence beginning in early June. While the male is exclusively glycophagous, the female also feeds on *M. pruinosa* nymphs and parasitizes the third, fourth and fifth instar nymphs of the flatid by laying an egg between the wing pads of its hosts. Within a few days after oviposition, the egg hatches and the emerging larva becomes visible as a cyst (thylacium) protruding laterally on one side of the host's thorax. The larva emerges from the host then spins a silky cocoon under the remains of the host, usually on the underside of leaves, where it completes its development (Guglielmino and Bückle, 2003). Some *N. typhlocybae* larvae enter diapause, overwintering and completing development

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in the spring-summer of the following year (monovoltine population). The remaining larvae immediately pupate and give rise to adults during the same year (bivoltine population).

Bivoltinism is of major importance for the effectiveness of biological control. Individuals from the bivoltine cohort that feed in August and September on planthopper nymphs that survived spring predation and parasitization contribute to increase the number of overwintering larvae and, consequently, the number of adults in the following year (Mazzon et al., 2001). Most of *N. typhlocybae* individuals currently released in Europe originated from a population collected in Connecticut (USA) and reared in Italy (Girolami, 1999). The population of *N. typhlocybae* in Pisa, originally collected in College Station (Texas) (Lucchi et al., 2002), has 80% bivoltinism, which is more than twice that of the Connecticut population.

The larva of *N. typhlocybae* spends most of its life inside a flat, oval cocoon with an internal elliptical larval chamber that completely envelops the larva and is surrounded by a silky layer (cocoon wall, Fig. 1). Usually cocoons spun by male larvae appear smaller than those of females, but constant and reliable morphological features suitable for gender discrimination, based on the size and/or shape of larva and cocoon, are not available for this species.

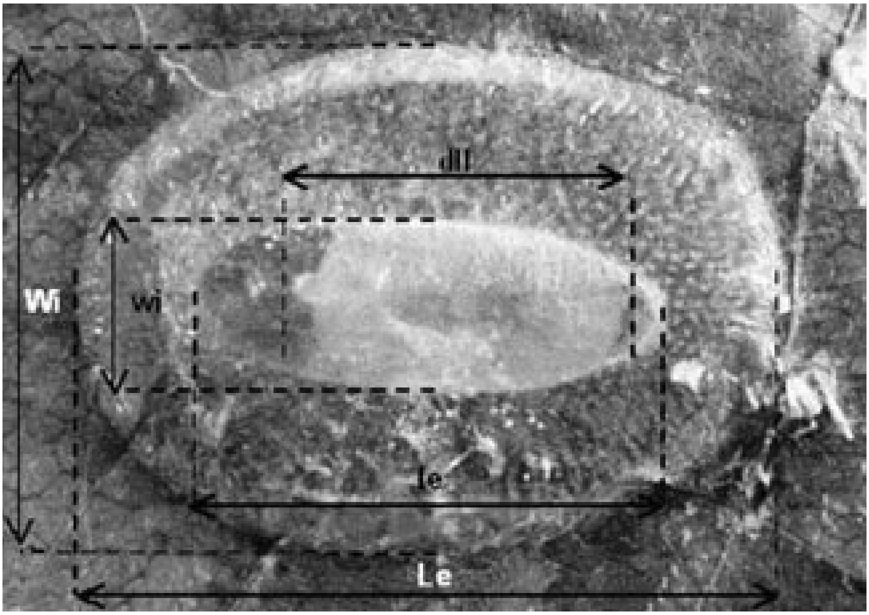

Fig. 1. Cocoon and diapausing larva of *N. typhlocybae.* Arrows represent the five measurements taken from each cocoon: length (Le) and width (Wi) of the cocoon wall; length (le) and width (wi) of the larval chamber and length of the diapausing larva (dll).

Morphometric studies on insects have dealt mainly with variation in adults (Wool and Hales, 1997; Heinz, 1998; Norry et al., 1999; Williams and Goodell, 2000; Hernández-Ortiz et al., 2004; Okada and Miyatake, 2004) and, in a few cases, immatures (Bernays and Hamai, 1987). In this study, a statistical multivariate approach was used to explore relationships among different morphological features (Sites and Willig, 2000; Sepp et al., 2004). We utilized multivariate analysis of variance (MANOVA) to evaluate differences among populations and between genders based on mensural characters of cocoons and diapausing larvae of *N. typhlocybae.*

METHODS

The study was carried out from August 2003 to July 2004 in the experimental field plot next to the Faculty of Agricultural Science building on the campus of the University of Pisa. A total of 790 overwintering larvae and cocoons of a population of *N. typhlocybae* derived from specimens collected in Texas (U.S.A.) was examined. Cocoons were obtained from parasitized nymphs of *M. pruinosa* fed on *Pittosporum tobira* L. (Pittosporaceae) potted plants located in a permanent open air cage. Most of the cocoons that were examined derived from parasitized 5th instar nymphs of the host by bivoltine females of *N. typhlocybae.*

In autumn, leaves bearing parasitoid cocoons were collected in plastic vials and left in the outdoor cages under ambient temperature and moisture conditions. In March, before the end of diapause, leaves with cocoons were transferred to individual vials and taken to the laboratory to be measured. Measurements were made using a micrometer on a Leitz Wetzlar (Germany) stereomicroscope. Five measurements of each specimen were taken: length (Le) and width (Wi) of the cocoon wall; length (le) and width (wi) of the larval chamber and length of the diapausing larva (dll, Fig. 1). After measurements were taken, leaves bearing cocoons were returned to the outdoor enclosure. Beginning at the end of March, each cocoon was examined weekly until development of the white pupal stage, when sex was assigned based on characteristics of the prothoracic legs and ocelli (Mazzon et al., 2000).

Statistical Analyses

Data were analyzed collectively using a one-factor multivariate analysis of variance (MANOVA) to test the effect of the "gender" factor on the measured variables. Wilk's Lambda was used to test the significance of MANOVA. Before MANOVA, all dependent variables were tested for intercorrelation and those that were significantly correlated were removed. One-factor analysis of variance (ANOVA) was utilized to assess which variable was significantly influenced by "gender." Linear correlations were applied among all the variables for the whole population as well as for males vs. females.

RESULTS AND DISCUSSION

Between the end of May and the beginning of June, all the individuals in the cocoons were in the stage of black pupae. Emergence of adult *N. typlocybae* began on June 7, 2004, and continued for two weeks (Fig. 2). This short emergence range is similar to that observed by Girolami and Mazzon (2001) for a Connecticut population of *N. typhlocybae.*

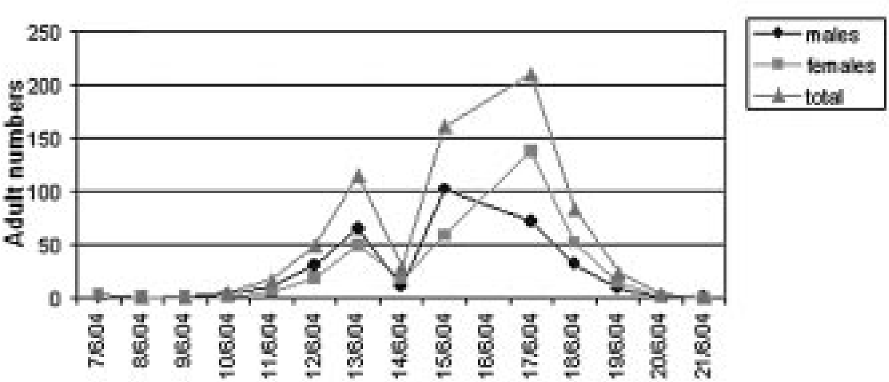

Fig. 2. Emergence times of the overwintering population of *N. typhlocybae* in 2004.

Of the 790 cocoons collected, 769 subsequently completed development (398 females, 371 males). Results of MANOVA for all morphometric traits indicate that the variables are influenced by "gender" (Wilks' Lambda = 0.190452; F = 648.65, $P \leq 0.001$; N = 769). The results of one-way ANOVA tests indicate significant differences between males and females in the larval mensural characters (Table 1). The variables that best characterize differences between the two sexes are the length of the larval chamber (le) and the length of the diapausing larva (dll) ($F_{le} = 2769.47$; $F_{dll} = 2221.51$; $P \leq 0.001$; N = 769).

The ratios between the mean values of females and males are 1.2 for Wi, 1.2 for wi, 1.3 for Le, 1.4 for le and 1.3 for dll. Thus, with reference to the cocoon wall, larval chamber and diapausing larva length, the values are lower in males than females, but the cocoon wall measures are highly variable, as indicated by the coefficients of variation of Wi and Le (20% and 10% in females and 20% and 20% in males, respectively). For this reason, the measurements of the cocoon wall are less reliable for accurate sex discrimination, as they are influenced by several factors. For example, *N. typhlocybae* larvae often construct cocoons near leaf veins which influence the final shape of the cocoon wall. This observation is also confirmed by percentile analysis graphically represented in the box plot (Fig. 3), where the variables "wi," "le," and "dll" show a narrower distribution in both sexes.

Table 1. One-way analysis of variance (ANOVA) on five morphometric traits (in mm) for the population of female and male cocoons of *N. typhlocybae*. For each variable DF = 1; all F values are significant ($P \leq 0.001$).

Variables	MEAN ± SD		F
	Females	Males	
Wi	4.1 ± 0.67	3.3 ± 0.66	283.57
wi	1.7 ± 0.14	1.4 ± 0.16	1077.15
Le	6.6 ± 0.63	4.9 ± 0.74	1189.67
le	4.4 ± 0.28	3.2 ± 0.38	2769.47
dll	3.3 ± 0.21	2.5 ± 0.28	2221.51

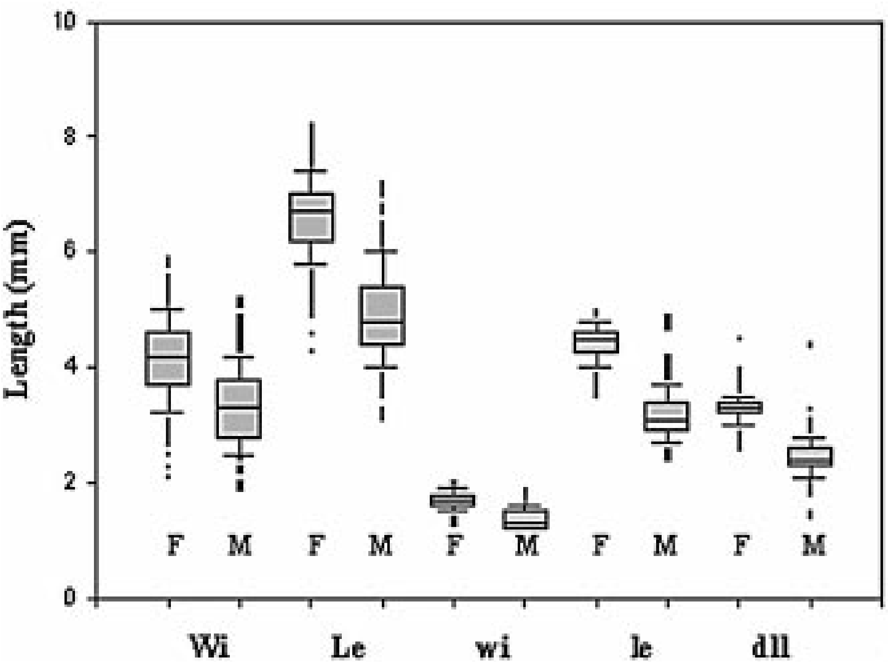

Fig. 3. Box and whisker representation of mean value of the five variables observed in females and males. For each variable, the top line represents the 90th percentile, the bottom line the 10th percentile and the box represents the 75th percentile (upper side), the 25th percentile (lower side) and the median (50th percentile, central line).

Correlations between each morphometric variable for the entire pool of data exhibited strong associations ($R^2 > 0.70$) for 6 of the 10 combinations (Table 2). Correlations between variables for males and females suggest a greater correspondence between the variables for males (7 of 10 with $R^2 > 0.40$) than females (3 of 10 with $R^2 > 0.40$, Table 3).

Data indicate that the morphometric approach is suitable for sex discrimination of the larval stages in *N. typhlocybae* using sizes of the cocoon wall, larval chamber and diapausing larva. Use of morphometric features allows recognition of gender from the time of cocoon spinning until almost one year prior to adult emergence, is easily and quickly done, and does not damage the developing larvae.

Table 2. Relationship among the morphometric traits in *N. typhlocybae*. R^2 is the coefficient of determination.

Correlations	**Equation**	**R^2**
Wi *vs.* Le	Y = 0.93X + 2.27	0.43
Wi *vs.* wi	Y = 0.21X + 0.76	0.48
Wi *vs.* le	Y = 0.60X + 1.57	0.42
Wi *vs.* dll	Y = 0.40X + 1.37	0.40
Le *vs.* wi	Y = 0.18X + 0.52	0.70
Le *vs.* le	Y = 0.59X + 0.40	0.82
Le *vs.* dll	Y = 0.39X + 0.62	0.76
le *vs.* wi	Y = 2.79X + 0.49	0.81
dll *vs.* wi	Y = 1.86X + 0.02	0.75
dll *vs.* le	Y = 0.64X + 0.43	0.87

Table 3. Correlations between cocoon and diapausing larva morphometric traits in the population of females (above diagonal) and of males (below diagonal). Numbers represent the coefficient of determination.

♂ ♀	Wi	Le	wi	le	dll
Wi	1	0.13	0.20	0.14	0.11
Le	0.33	1	0.16	0.47	0.30
wi	0.40	0.56	1	0.43	0.33
le	0.32	0.61	0.67	1	0.47
dll	0.26	0.45	0.48	0.53	1

In conclusion, cocoon wall measurements can certainly be adopted for applied research. On the other hand, as the lengths of the larval chamber and diapausing larva are strongly associated with gender, we suggest using these variables for taxonomic studies on *N. typholocybae* and related taxa.

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