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Does diet affect morphological parameters of *Neoseiulus tunus* (De Leon) (Acari: Phytoseiidae)?

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Original research

ABSTRACT

Neoseiulus Hughes is one of the most diverse genus of Phytoseiidae, with approximately 400 described species. Among them, *Neoseiulus tunus* (De Leon) is very frequent and abundant in the Neotropical region. Although considerable amount of data about morphological variation of phytoseiid mites are available, the possible causes of intraspecific variations are still poorly understood, being diet and feeding habit usually reported as the main drivers. The present study investigated the influence of three different diets on morphological traits of taxonomic interest of *N. tunus*, aiming to verify whether diet is a determinant factor in the morphological pattern of this species and which structures are most affected by them. Our results show that based on the diet treatment, populations of *N. tunus* may be differentiated based on the size of morphological traits. All treatments differ from stock population and among them, except between populations feeding with *Aculops lycopersici* (Massee) (Eriophyidae) and *Tetranychus urticae* (Kock) (Tetranychidae). The widest range occurred on the dorsal setae, macrosetae of leg IV, width of the dorsal shield, distance between the bases of the genital's setae and posterior width of the ventrianal shield. Most of the dorsal setae responded to the treatment with significant variation, being the longest lengths observed when the *A. lycopersici* was offered as diet. Considering the other two diets, the variation does not present a clear increasing or reducing pattern.

Keywords biological control; feeding habit; phenotypic plasticity; taxonomy

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Introduction

Environment heterogeneity may affect plastic characteristics of animals in different ways (e.g. age and size at maturity) (Pigliucci 2001). Also, Mikolajewski *et al.* (2005) points out that the size of many mature organisms is clearly modified as a response to the food limitations and availability.

Morphological intraspecific variation has been reported in several groups of mites, being quite common for species and genus of Phytoseiidae. Furtado (1997) verified the occurrence of small variations of some structures, especially the length of setae when they are reared providing different diets. Lofego (1998) analyzed morphological parameters of around 50 phytoseiid species and found that some of them showed substantial variation among populations from different regions of Brazil, such as highlighted by the deviation up to 100% in the length of some setae from the dorsal shield of *Euseius alatus* (DeLeon). Tixier *et al.* (2008) studying 10 populations of *Neoseiulus californicus* (McGregor) from eight countries (Brazil, Chile, France, Greece, Italy (Tuscany), Italy (Sicily), Japan, Spain and Tunisia), pointed out that

most variables had significant differences among the studied groups, and also proposed a statistical approach to study the intraspecific variations of characters used in species diagnosis (Tixier 2012). Several explanations are provided to explain such variation, the body size and biogeographic or biotope origin of the populations due to the environmental characteristics and their reproductive isolation.

The influence of the feeding habit on morphological traits of Phytoseiidae mites has been suggested in several publications (Croft *et al.* 1999; Tixier *et al.* 2003, 2004, 2006). However, more information is still needed to determine the dimension of this impact. Phytoseiidae (Acari: Mesostigmata) is a family of predatory mites, including some species used in biological control programs worldwide (McMurtry and Croft 1997; Gerson *et al.* 2003; Hoy 2011; McMurtry *et al.* 2013). This family comprises 2,866 described species included in 94 genera (Demite *et al.* 2021).

Different studies report that body size, weight and development characteristics of phytoseiids differ among species and between populations of a same species, depending on the quality and type food that is provided, such as pollen, preys or even artificial diets (McMurtry and Scriven 1966; Badii *et al.* 1990; Croft *et al.* 1999; Goleva *et al.* 2013, 2015; Nguyen *et al.* 2014; Lopes *et al.* 2018). Most of phytoseiid species are generalist predators, they can feed wide range of resources such as phytophagous mites (e.g. Tetranychidae, Eriophyioideae, Tenuipalpidae and Tarsonemidae), small insects (e.g. *Scirtothrips dorsalis* Hood, Thripidae and *Bemisia tabaci* Gennadius, Aleyrodidae), nematodes, fungi and even plant tissues such as pollen and exudates (Gerson *et al.* 2003; McMurtry *et al.* 2013; Amaral *et al.* 2018). The wide range of feeding items consumed by phytoseiids allows us to ask whether different diets used by the same species may result in morphological variation.

The present study aims to determine whether the diet causes variation in the morphological traits of the phytoseiid *Neoseiulus tunus* (De Leon), and which parameters are more affected by three distinct diets.

Material and methods

Origin of specimens and rearing techniques

Specimens of *N. tunus* in the experiment were obtained in samplings on *Trichilia casaretti* L. (Meliaceae) in a forest fragment located in the municipality of Icém – SP, Brazil (20°20'48" S; 49°15'49" W), in the year of 2014. *Neoseiulus tunus* is a widespread species, easily found in native and cultivated plants in the northeastern of São Paulo state. This species is barely studied for biological control purposes, although it was already observed consuming *Tetranychus urticae* in field and laboratory. So, due to the potential use for biological control and being widespread in the studied area, this species was elected for the study.

They were kept in the laboratory starting a stock colony at breeding units composed by a vinyl floor plate (Paviflex®) placed on a foam, inside a tray containing distilled water. The edges of the plates were framed by cotton in contact with the foam, consequently both (the foam and the cotton) remained continuously soaked by the water kept in the tray. These breeding units were placed in an incubator chamber (B.O.D.) at 25 ± 1 °C, relative humidity of 60 ± 10% and photoperiod of 12 hours. The mites from the stock colony were feeding with diverse food items, including cattail pollen (*Typha angustifolia* L.) (Typhaceae), added each 2 days, supplied with recently emerged individuals and eggs of *Tyrophagus putrescentiae* (Schränk) (Acaridae) who served as an alternative food source for phytoseiids. Throughout the development of the colonies, *Aculops lycopersici* (Massee) (Eriophyidae) and *Tetranychus urticae* (Kock) (Tetranychidae) were offered as complementary feeding resource in their diet. The populations of the stock colony were transferred to new breeding units at least once a month or whenever necessary, thus avoiding presence of accumulated debris, fungi and excretes that could damage the feeding and reproduction of these mites.

The cattail pollen was continuously collected and according to its availability in the municipalities of São José do Rio Preto - SP and Bady Bassitt - SP, brought to the acarology laboratory and stored / frozen in vials.

The colonies of phytophagous mites *A. lycopersici* were maintained on *Petunia* sp. and *Lycopersicon esculentum* Mill, both plants of the family Solanaceae, while *Tetranychus urticae* was maintained on *Canavalia ensiformes* (L.) Decandolle (Fabaceae). The host plants were sowed in greenhouses and then kept in the laboratory condition while the experiment was carried on.

Experimental design

All experiments were conducted in experimental units identical to the rearing units of the stock colony in the laboratory.

Three different treatments (diets) were set to analyze morphological variation of *N. tunus*: cattail pollen (*Typha angustifolia* L.) (Typhaceae), *T. urticae* and *A. lycopersici*. For each treatment we established seven new colonies (equal to seven breeding units), which were started with 10 adults and possibly fertile females taken from the original stock colony kept in the laboratory. Forty-nine (49) females were randomly withdrawn from the stock colony and mounted in microscopy slides to evaluate the morphology of the initial population, for posterior comparison with treatments. The population of *N. tunus* of each treatment received only one type of food resource (cattail pollen, *T. urticae* and *A. lycopersici*) for the time period of 60 days and were kept in an incubator chamber (B.O.D.) at 25 ± 1 °C, with relative humidity of $60 \pm 10\%$ and photophase of 12 hours. In the end of this period, seven adult females were removed from each breeding unit (totaling 49 specimens) and mounted on microscopy slides for the measurement of all the structures and parameters. Every two days the food item was replaced in each colony to ensure the full development of the mites. The water in the tray was replaced whenever necessary to maintain the moisture of the foam and cotton.

Morphological analysis

The chaetotaxy nomenclature followed the system proposed by Rowell *et al.* (1978) and Chant & Hansell (1971) for the dorsal and ventral shields, respectively. All measurements are given in micrometers (µm).

Several structures were analyzed as length of the dorsal shield (LDS), from the base of the j1 setae to the posterior extremity; width of the dorsal shield (WDS) at the level of the setae s4; distance between the bases of the setae ST2 (sternal) (DBST2) and between the bases of the setae ST5 (genital) (DBGS); anterior width of the ventrianal shield (AWVS) at the level of the setae ZV2; posterior width of the ventrianal shield (PWVS), at the level of the para-annals setae; length of the ventrianal shield (LVS) (Figure. 1); length of the dorsal setae j1, j3, j4, j5, j6, J2, J5, z2, z4, Z5, Z1, Z4, Z5, s4, S2, S4, S5, r3 and R1; length of the macrosetae Sge IV (genu IV), Sti IV (tibia IV) and St IV (tarsus IV); length of the cervix of the spermatheca. The measurements were made using an optical microscope with phase contrast (Zeiss Scope A.1), provided with a millimeter ocular lens.

The voucher material was deposited in the collection Acari (DZSJRP) – <http://www.splink.cria.org.br>, of the Department of Zoology and Botany, Universidade Estadual Paulista (UNESP), São José do Rio Preto, São Paulo, Brazil.

Statistical analysis

In order to compare the effect of each treatment in the set of 30 morphological traits we performed a permutational analysis of variance (PERMANOVA, 9999 permutations) with a pairwise comparison. Specific analysis of variance (one-way ANOVA) was also performed for each parameter followed by a post hoc Tukey's test ($p < 0.05$) considering the variation among treatments. The raw data from the measures were transformed considering Euclidian distance,

Figure 1 *Neoseiulus tunus* (Denmark & Muma): Female: A – positions of measures established for dorsal shields; B – Positions of the measures established for the ventral shields. Adapted from Cavalcante *et al.* 2017.

and were defined 1000 permutations for each test. Therefore, two multivariate statistical analyses were also performed on the 30 quantitative traits. We run a principal component analysis (PCA) to reveal any difference in the morphological variation among the specimens from different treatments and to indicate the contribution of each morphological parameter to each axis of analysis. Thereafter we performed a non-metric multi-dimensional scaling (NMDS) to identify which morphological traits that contributed the most to the morphological differentiation (based on R^2 values) among the populations of *N. tunus* allowing to distinguish them for each treatment. The analyses were performed using vegan package (Oksanen *et al.* 2019) in R (R core team 2020).

Results

The permutational analysis of variance (PERMANOVA) including all the morphological traits and in relation to the treatments, indicated a considerable high level of significance for the diet test with a ($F_{3,183} = 9.13$, $p = 0.001$). Considering the pairwise comparison *a posteriori*, the morphologic traits of each treatment were distinct from the stock population, and varied

among each other (*A. lycopersici* x Stock population $F_{3,183} = 15.31$, $p=0.002$; Pollen x Stock population $F_{3,183} = 11.79$, $p=0.02$; *T. urticae* x Stock population $F_{3,183} = 13.061$, $p= 0.012$; *T. urticae* x pollen $F_{3,183} = 6.29$, $p= 0.012$; *A. lycopersici* x pollen $F_{3,183} = 6.17$, $p= 0.013$), unless the treatments feed on *A. lycopersici* and *T. urticae* that did not differ from each other ($F_{3,183} = 1.37$, $p=0.273$). Those results corroborated the multivariate analysis, since they indicated that most specimens may be grouped according to each treatment, however there were a higher overlap mainly between the treatments of phytophagous species, *A. lycopersici* and *T. urticae* (Figure 2). On the other hand, the specimens from stock population and from pollen treatment represented the most distinct populations.

The first two axes of PCA analysis explained about 70% of total morphological variation (PC1: 63.36%; PC2: 6.15%), being the first mainly associated to the dorsal setae (such as z4, Z5, j2, z2, LDS, j6, j1, j3, j6 and Z4), while the second was compound by ventral shields length and spermatheca (DBST2, DBGS, WDS and Spermatheca) (Figure 2). The partial overlap of specimens from distinct treatment indicate that these populations may not be totally separated and distinguished by their morphological traits, despite there are some evident effects of the feeding habit over some characteristics.

The NMDS analysis also presented similar results from PCA, with a supplementary information about the main morphological traits that allowed to distinguish the specimens among treatment (Table1). These results indicate that traits related to ventral shields and dorsal setae are the main drivers of morphological differences among those population (Table 1).

Among the thirty variables, eighteen of them showed significant differences between the diets. (WDS, DBGS, PWVS, Sge IV, Sti IV, St IV, j3, j2, z2, z4, z5, Z1, Z4, s4, S4, S5, r3 and R1).

For the morphological traits corresponding to shields (WDS, DBGS and PWVS), the measurements of these structures were significant higher when the mites were fed with phytophagous mites than with pollen (Figure 3), despite they did not differ between each other.

Considering the measures of macrosetae from the legs, those of the genu IV and tibia IV presented longer dimensions in the treatments with pollen and *A. lycopersici* when compared with the stock population (Figures 4. a & b). The macroseta of the tarsus IV was significantly lower only when the prey provided was *T. urticae* (Figure 4.c).

The effect of diet type was observed in most of the dorsal setae. The longest setae size was remarked when *A. lycopersici* was offered as food item, since this pattern was observed for 9 of 12 dorsal setae studied (Figure 5). Considering the other two treatments, the variation in the setae size did not present a clear pattern (Figure 5).

Table 1 Main morphological traits related to the ordination analysis, according to their R^2 . There are shown only significative ($p<0.05$, and with $R^2 > 0.65$).

Trait	NMDS1	NMDS2	R^2	P
WDS	-0.6704334	0.741969731	0.95	0.001
LDS	-0.8147244	-0.579848419	0.92	0.001
LVS	-0.9810883	0.193560876	0.79	0.001
Z5	-0.9147859	-0.403938993	0.73	0.001
J2	-0.9999122	0.013247674	0.73	0.001
j1	-0.7394630	-0.673197215	0.69	0.001
S2	-0.9990862	-0.042740748	0.68	0.001
r3	-0.9374295	0.348175118	0.68	0.001
z2	-0.9604262	0.278534624	0.66	0.001

Figure 2 Principal component analysis (PCA) of 30 morphometric characters measured on females of *N. tunus* populations from distinct treatments. Individuals plotted against their values for the first two principal components. Each seta represents a morphological trait, and each polygon a distinct treatment.

Despite the differences observed in the setae length among the treatments, it is possible to observe that the range of measurements obtained are within the spectrum of length obtained by specimens of *N. tunus* collected in the field from different regions by Cavalcante *et al.* (2017); Moraes *et al.* (2000); Guanilo *et al.* (2008a, b) (Table 2).

Figure 3 Variation in the size of the ventral shield of *N. tunus* fed different diets: WDS-width of the dorsal shield; DBGS – distance between the bases of the genital setae and PWVS – posterior width of the ventrianal shield. *Different letters show statistical significance between treatments at $p < 0.05$.

Figure 4 Variation in the length of macrosetae of the leg IV of *N. tunus* among different treatments: A – genu; B – tibia; C – tarsus.

Discussion

Variations in the morphological traits are usually observed in the populations of phytoseiid mites. According to our results, part of these variations may be related to the feeding resource available. Some food items might have the tendency to induce the increasement of the structure lengths, such as observed for *A. lycopersici*; or influencing either reduction or increasement other structures, as observed for *T. urticae*.

Some of the most significant variations were observed in the length of the dorsal setae and the in the macrosetae of leg IV. A similar pattern was found by Noronha (2002) in an experiment carried out on *Euseius citrifolius* (Denmark & Muma). Abou-Setta *et al.* (1987) studding *Euseius mesembrinus* (Dean) from Florida and Texas (USA) pointed out some morphological range in the setae according to geographic and diet effect, also noticed that such variation occurred in organisms collected in the field although it was not observed in laboratory conditions.

Hoying *et al.* (1977) reported morphological variations among eight populations of *Galendromus longipilus* (Nesbitt) and 12 populations of *Galendromus occidentalis* (Nesbitt) collected in different regions of the United States of America. In this study they tracked the main variation associated to the length of the peritreme and length of the dorsal shield. In the present study we did not evaluate peritremal shield, but the length of the dorsal shield (LDS)

Figure 5 Variation in the length of the dorsal setae of *N. tunus* fed according to the treatments.

had no variation considering distinct diets. However, the width of the dorsal shield (WDS) was a character that changed in dimension when the resource offered was phytophagous mites (*A. lycopersici* and *T. urticae*). Few studies reporting morphological variations in the dorsal shields,

Tixier *et al.* (2008) highlighted that specimens from Brazil had higher values for the length of the metapodal plate 1 (not measured in the present study) and width of the ventrianal shield. In our experiment, some variation was observed only for measurements of the posterior region of the ventrianal shield, and only when phytophagous mites were provided as food resource.

Goleva *et al.* 2015 shed a light about the tendency of variation in the in morphological traits in diverse Phytoseiidae populations, considering that limitation and quality of the food supply are strictly linked to the development, longevity, and reproduction of predatory mites. The

Table 2 Measurements (mean values and minimum-maximum range) of specimens according to three treatments (pollen, *A. lycopersici* and *T. urticae*): compared to measurements of the holotype and minimum-maximum range of other native populations in South America.

Characters	Treatments			<i>N. tunus</i> ¹ De Leon (1967)	Native populations of South America ²
	Pollen	<i>A. lycopersici</i>	<i>T. urticae</i>		
WDS	286 (275-300)	286 (267-300)	287 (267-297)	288	270-315
LDS	177 (167-187)	180 (165-187)	180 (152-192)	166	150-192
j1	22 (13-25)	23 (16-26)	23 (15-26)	23	19-27
j3	27 (23-29)	27 (23-30)	27 (20-30)	28	19-31
j4	15 (13-20)	15 (13-16)	15 (10-17)	13	11-17
j5	14 (13-16)	15 (13-16)	15 (13-21)	16	11-18
j6	20 (17-22)	20 (16-23)	20 (16-23)	20	14-24
J2	21 (18-23)	22 (19-25)	22 (19-24)	20	16-28
J5	8 (5-9)	8 (5-9)	8 (5-10)	7	7-10
z2	21 (20-24)	22 (20-25)	21 (17-24)	20	19-27
z4	29 (27-31)	30 (27-32)	30 (25-32)	30	23-37
z5	16 (15-19)	16 (12-18)	15 (13-18)	16	11-19
Z1	25 (23-25)	26 (24-29)	26 (22-28)	24	21-32
Z4	43 (40-45)	44 (41-47)	43 (39-47)	43	39-54
Z5	69 (56-73)	69 (60-76)	68 (59-74)	64	63-80
s4	37 (35-38)	39 (35-42)	38 (35-42)	36	33-44
S2	34 (30-37)	34 (30-38)	34 (31-39)	31	29-43
S4	20 (18-22)	21 (19-23)	21 (18-23)	19	17-30
S5	13 (11-14)	13 (8-15)	12 (8-14)	12	11-20
r3	24 (22-26)	24 (18-26)	23 (14-26)	24	20-30
R1	16 (15-17)	16 (15-18)	16 (15-18)	14	14-20
DBST2	70 (68-75)	71 (67-75)	70 (62-74)	62	60-70
LVS	103 (93-109)	102 (88-108)	102 (89-110)	98	95-117
AWVS	70 (62-75)	72 (66-76)	72 (74-78)	70	63-80
PWVS	57 (53-60)	57 (51-69)	57 (50-62)	54	51-65
C. Spermatheca	10 (8-12)	10 (8-11)	10 (8-13)	6	6-11
SgeIV	22 (18-24)	21 (15-24)	21 (15-23)	22	16-24
StiIV	20 (17-21)	19 (15-20)	19 (17-21)	19	15-23
StIV	32 (29-34)	32 (23-35)	31 (29-34)	32	23-36

¹Holotype: *Neoseiulus tunus* (De Leon, 1967): 29; Upper Saint John s Road, Tunapuna, Trinidad, on *Psidium guajava* .

²Cavalcante et al. (2017); Moraes et al. (2000); Guanilo et al. (2008a, b).

study highlights the influence of different types of pollen (*Betula pendula* Roth, *Typha latifolia* L., *Pinus sylvestris* L., among other sources) and moth eggs (*Cydia pomonella* L. (Lepidoptera, Tortricidae) mainly on the body weight of the mites, linking body weight with reduction or increase in the body sizes due to the diet which the phytoseiids were subjected to.

Our data supports that diet type is a key element driving the shifts of morphometric traits in the populations of *N. tunus*. Understanding these variations is important since the morphological determination of a species is based in the entire range of its traits and structures. The particular determination of this range can only be done with laboratory experiments at controlled conditions. This way, we can ensure that a certain range in the morphological variation may correspond in fact to the intraspecific variation and not due to contamination by another species in a given population. In this context, there is also a necessity of more efforts to investigate the effects of diets on other species of the family Phytoseiidae, in order to better understand the causes of intra-specific variations and consequently avoid errors of identification.

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