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Morphological Changes in Some Taxonomic Characters of Laboratory-Reared *Neoseiulus idaeus* (Denmark and Muma)

G. S. Mwansat

Department of Zoology, University of Jos, Jos, Nigeria

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ABSTRACT: Microscopic measurement of 29 morphological features of female *Neoseiulus idaeus* (Denmark and Muma) were made using the Leitz phase contrast compound microscope on both feral and colonized strains. The width of the dorsal shield, lengths of setae j3, j5, z1, s4 and s5 on the dorsal shield, the distances between setae ST1 andST3, and the pair of ST2 on the sternal shield, the pair of G setae on the genital shield and the length of the ventrianal shield were significantly longer (P.0.05) for the feral strain females than for the colonized stain females. Conversely, eleven taxonomic characters of the feral stain namely, the dorsal shield setae j1, j5, j6, j2, z4, Z4, r3, and R1, macroseta on tarsus of leg IV, the anterior and posterior widths of the ventrianal shied were significantly shorter (P, 0.05). The elongation of some the dorsal shield setae of the colonized strain was attributed to adaptation for feeding on variation (%) obtained for the majority of the parameters in the colonized strain suggested greater genetic diversity in the feral population and/or convergence of external features due to laboratory selection predator be reared on its original primary prey and the original host plant under near natural condition to achieve control when released in the field.

Key Words: Cassava (*Manihot esculenta*); Cassava pests; Cassava green mite; Cassava mealybug; Insect taxonomy; Morphological characteristics; *Neoseiulus idaeus*.

Introduction

Cassava (*Manihot esculente* Crantz (Euporbiaceae) is the main staple food for about 200 million Africans, representing 80% of the population, from 31 countries on the continent (1). Although cassava ranks seventh in production among the major food of the world (2), its importance has been widely recognized only within the last decade (3).

Until 1971, cassava production in Africa was free of a wide of range of biological constraints especially pests and diseases found elsewhere in the world.

Reports of the accidental introduction of two Neotropical pests of cassava into Africa enhanced entomological focus on cassava after 1971 (4,5). The spread in Africa of the introduced cassava green mite (CGM), *Mononychellus tanajoa* (Bonder) (Tetranychidae), reported for the first time in Uganda in 1971 (6) and that of the cassava mealybug (CM), *Phenacoccus manihoti* Mat-Ferr. (Homoptera: Pseudococcidae), reported for first time in Zaire in 1973 (7) was rapid, causing severe damage to the crop. CGM has now been reported in most of the cassava-growing regions of Africa (8,9).

Control strategies suggested for CGM include cultural, chemical, host plant resistance and biological control. The classical biological control method (the use of introduced natural enemies to keep an exotic pest below its economic injury level) has been recommended particularly as CGM is an exotic pest and local predators were found to be inefficient (9,10).

Therefore, based on extensive surveys of CGM natural enemies in its region of origin in the neotropics, seven phytoseiid species have been selected for introduction into Africa for a continent–wide biological control of the pest by the Biological Control Programme of the International Institute of Tropical Agriculture (llTA), Nigeria (11,12). The phytoseiids presently in culture at IITA's biological control facilities now located in Cotonou, Republic of Benin are *Neoseeiulus ideaus* (Denmark and Muma) (first imported, May 1983), *N. anonymus* (Chant and Baker), *N.chilenensis* (Dosse), *Gelendromus annectens* (DeLeon), *Tyhlodromalus limoniclis* (Muma) (13) (J. S Yaninek and A. Klay, personal communications). To date, some 5.5 million individuals of these species have been laboratory–reared and released in several locations in some cassava growing areas in Africa (14).

In the process of establishing a population in the laboratory, a sample of arthropods were taken from a much larger feral population. All subsequent genetic changes in the new population will be made from the lower genetic variation present in the now closed founder sample (15). The force of natural selection has been shown to affect the distribution of gene frequencies in a newly established laboratory population. Since environmental condition in the laboratory are undoubtedly different from those encountered by the feral population, certain individuals not favoured in the natural condition may become more fit in the laboratory.

The numbers of individuals responding favorably to the new environment may be very small (personal observations). Thus natural selection for fitness in the laboratory should increase the frequency of certain genotypes, while decreasing the frequency of others (15,16). This selection had in several cases affected the behavioural, reproductive and morphological characteristics, induced genetic recombination and mutation of the colonized population and has raised several questions concerning the quality, performance, nutrition and rearing methods of such laboratory organisms (17). Improvement of quality in laboratory mass-reared biological control agents (predators, parasites and pathogens) can be assessed using the various inherent characteristics usually considered when choosing them as biological control agents. These characteristics include high searching capacity, high degree host specificity, good synchronization with host, high reproductive rate and high degree of fitness and adaptability to a wide range of ecoclimatic conditions (18,19). In order to minimize condition leading to genetic and morphological drift, new strains of field-collected individuals are periodically added to the laboratory stock (14). *N. Idaeus* which was shown to possess desirable predatory attributes (20,21) (along with other exotic phytoseiid species) has been released in many countries but has shown no appreciable impact on the pest and had not been released in appreciable numbers in follow-up surveys in successive seasons (20,22,23). Having been reared for some time in the laboratory before release, the quality of this predator becomes suspect in the face of these problems.

The Colombian biotype of *N. idaeus* was chosen as candidate species for this classical biological control of *M. tanajoa*. The first consignment collected at the Centro Internacional de Agricultural Tropicale, Cali, Colombia arrived at IITA in May 1983 via the Institute of Biological Control, London for quarantine procedures. With mean generation time of 16.5 days (11), this population, termed the colonized strain in this study, had undergone approximately 70 generations in the laboratory at the beginning of this work. The impact of releases of the Colombian biotype of *N. idaeus* originally made in East Africa and Subseqently in other areas of Africa where CGM is pest have not been encouraging (20,23,24). One possible explanation is that the laboratory biotype has undergone a series of selection, which rendered the population ineffective as a biological control agent. Many morphological characters have ben used in phytoseiid taxonomy. These include the number and placement of setae on the dorsal and ventral shields, the setation of the legs especially macrosetae and the selection of the genua, the shape of the ventrianal shield and the shape of the female spermatheca.

Other features include the shape of the male spermadactyI structure the dentition and nature of the chelicerae, the number and placement of the setae in the r-R series, and the length and nature of the peritremes (25,26,27). In addition to these traditional methods in studying phytoseiid taxonomy, numerical texonomy has also been used (28). The objective of this study, therefore, was to see whether there were morphological changes in the taxonomic features of the adult female.

Materials and Methods

(a) Preparation of clearing and mounting media.

The clearing agent, Lactophenol, was prepared in a 250ml beaker with the following ingredients added in sequence:

Lactic acid:	50ml
Phenol Crystals:	25g
Distilled Water:	25ml

The solution was then filtered through cheesecloth. Hoyer's medium was used as the permanent mountant. It was prepared by mixing in a 250ml beaker the following ingredients in sequence with a stirring rod:

Distilled water	50ml
Gum arabic (amorphic):	30ml
Chloral hydrate	200ml
Glycerine:	20ml

All solid materials were completely dissolved before successive reagents were added. It was necessary to heat the liquid slowly to about 50^{o} C to accelerate the dissolution of the gum arabic. The solution was filtered though a clean cheesecloth to remove bit of wood and other impurities from the gum arabic.

(b) Preparation of permanent microscopic slides of Neoseiulus idaeus females

A cohort of 100 3-4 days old adult females of each strain of *N. Ideaus* were killed in 70 percent ethyl alcohol in a glass vial. They were placed individually in another glass vial containing the clearing agent lactophenol, a solution effective in macerating internal tissues of preserved mites with little or no damage to the exoskeleton (29). Mites were left in the clearing agent for 24 hours at room temperature. Clearing was terminated by rinsing mites in distilled water several times in a glass petri dish until the lactophenol/water (cloudy) interface was removed.

A drop of Hoyer's medium was placed in the center of a precleaned 25 x 75 mm microscope slide. The specimen of a single female was lifted from the petri dish with a fine camel hair brush size 000 onto the mounting medium on the slide. The mite was directed to the bottom of the medium with a fine pin probe such that the gnathosoma is oriented downwards on the vertical axis. With the aid of clean pair of forceps, a 15mm coverslip was applied just near the edge of the droplet of Hoyer's medium and allowed to fall gently into place. Final orientation of the specimen was accomplished under the dissecting microscope by applying a gentle pressure with the aid the forceps on the coverslip surface. Mounted specimens were fixed for 24 hours on a "Kaiser" dryer plate at 35^{o}C to expel air bubbles. Further fixing and drying were accomplished by incubating specimens for seven days at 50^{o}C in a laboratory oven. A ring of the red insoluble sealant "Nigerlux Picin Pack" was applied around the outside margin of the coverslip sealing it to the slide surface.

c) Microscopic measurements of morphological features

Twenty-nine (29) morphological features of the mounted female were measured using the Leitz phase contrast compound microscope at a magnification of 200 with a fitted micrometer eyepiece with a scale of one division representing 3.1µm. They were dorsal shield length (DSL), dorsal shield width (DSW), length of dorsal seta j1 on dorsum, length of dorsal seta j3 on dorsum, length of dorsal j4 on dorsum, length of dorsal seta j5 on dorsum, length of dorsal seta j6 on dorsum, length of dorsal seta J2 on dorsum, length of dorsal seta J5 on dorsum, length of median seta z2 on dorsum, length of median l seta z4 on dorsum, length

A. Ventral side of *Neoseiulus idaeus*.

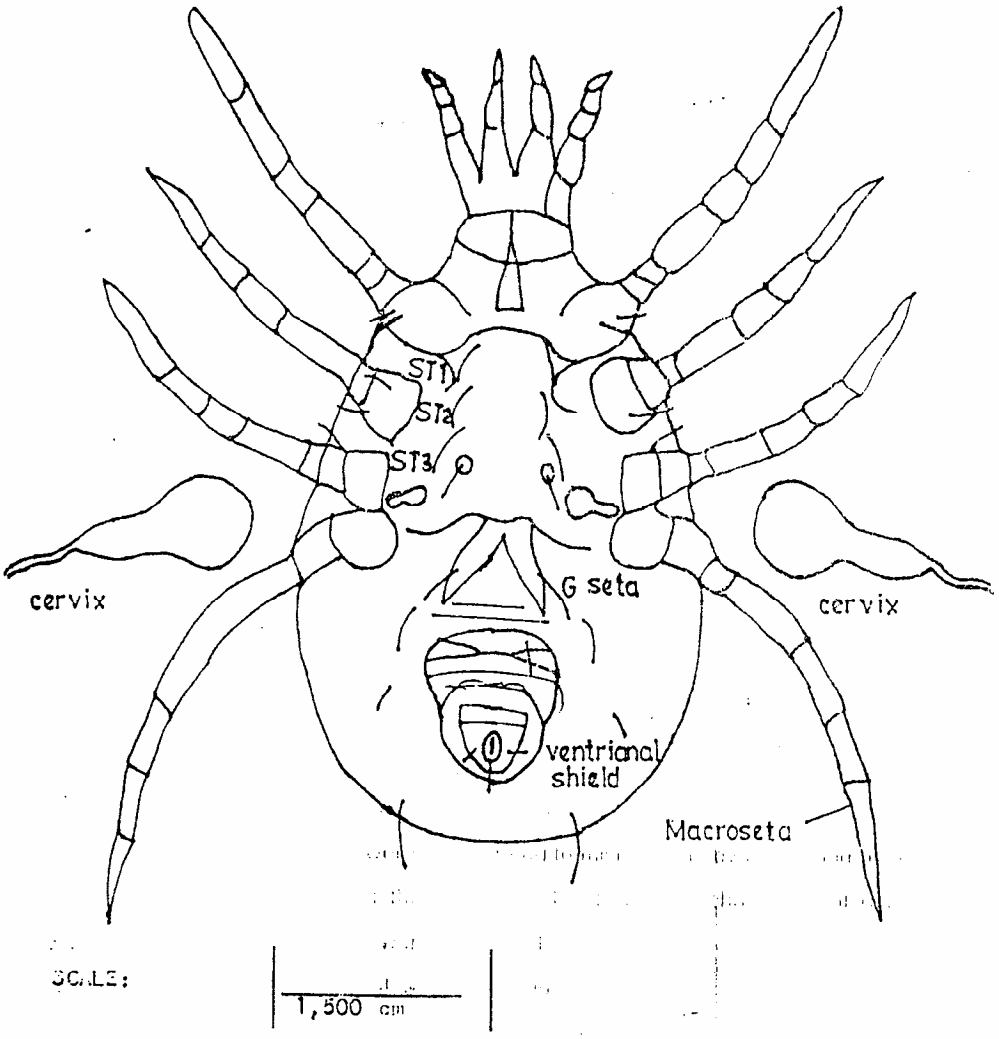

Fig. 1a: Taxonomic features of female *Neoseiulus idaeus*.

B. Dorsal side of *Neoseiulus idaeus*.

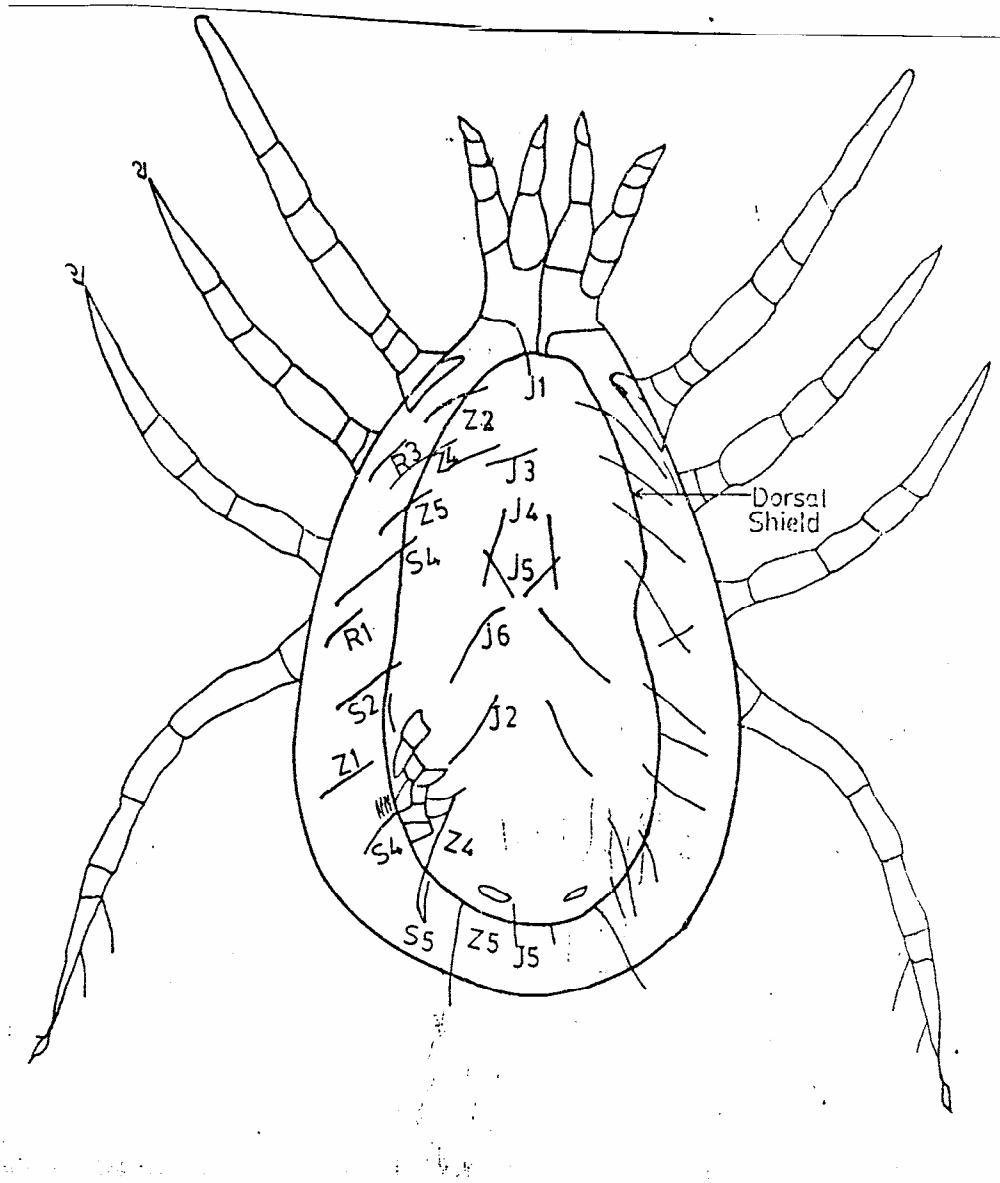

Fig. 1b: Taxonomic features of female *Neoseiulus idaeus*.

of median seta z5 on dorsum, length of median seta z1 on dorsum, length of median seta z4 on dorsum, length of median seta Z5 on dorsum, length of prolateral seta s4 on dorsum, length of sublateral seta S2 on dorsum, length of sublateral seta S4 on dorsum, length of sublateral seta S5 on dorsum, length of seta r3 on dorsum,length of lateral seta R1 on dorsum, length of macroseta on tarsus of leg IV,distance between sternal seta 1and 3 on the sternal shield (ST1-ST3), distance between the pair of sternal seta 2 on the sternal shild (ST2-ST2), distance between the pair of genital seta (G-G)anterior which of the ventrianal Shield (VAS-A),posterior width of venterianal shield (VAS-P) and length of the ventrianal shield (VAS-L).

Data collected were transformed to micrometers by multplying by a factor of 3.1. The ratios of the following closely related characters of dignostic importance of the mite were calculated:

Length of dorsal setae j4 and j5 on dorsum

Length of dorsal setae Z5 and J5 on dorsum

Length of dorsal setae r3 and R1 on dorsum

Length of dorsal setae Z1 and S4 on dorsum

Dorsal shield length (DSL) and dorsal shield width (DSW)

Distance between sternal setae 1 and 3 (ST1-ST3) and the pair of sternal setae 2 on the sternal shield (ST2-ST2)

Anterior width (VAS-A) and lateral length of the ventrianal shield (VAS-L).Difference in these ratios between the feral and colonized strains is hypothesized to denote morphological changes during colonization. A one-way ANOVA at 5% was used to test significance between means, and ratios of both treatments. Positions of these features in N. idaeus were as shown in Fig.1

Results

There were considerable differences in length of a majority of the morphological features measured (Table 1). The width of the dorsal shield, lengths of setae j3, j5, Z1, S4 and S5 on the shield, the distances between setae ST1 and ST3, and the pair of ST2 on the sternal shield, the pair of G setae on the genital shield and the length of the ventrianal shield were significantly longer ($P < 0.05$) for the feral strain females than for the colonized strain. Eleven characters were significantly shorter ($P < 0.05$) on the feral strain than on the colonized strain. These were dorsal shield setae j1, j5, j6, j2, z4, Z4, R3, and R1; macroseta on leg IV and the anterior and posterior widths of the ventrianal shield. No changes occurred in length of the dorsal shield, setae j4, z2, z5, Z5, s4 and S2 on the dorsal shield and length of the cervix of the spermatheca. Although there was considerable amount of overlap in the measurements of these features for both stains, they were move variable in the feral strain than in the colonized strain.

Table 2 shows the ratios of some of the closely related diagnostic characters of feral and colonized strains of N. ideas. The ratios between Z5/J5, r3/R1 DSL/DSW and VAS-A/VAS-L were significantly greater ($P < 0.05$) for the colonized strain than for the feral strain. The ratio between j4and j5 was signifiicantly greater ($P < 0.05$) for the strain (1.02) then for the colonized strain (0.98). There were on significant difference ($P = 0.05$) in the ratios Z1S4 and ST1-ST3/ST2 between the two strains.

Discussion

The lengths of feral and colonized *N. idaeus* morphological features were remarkably similar to those reported for the type specimen (30) and specimens reared at various temperatures (31). The type had a body length of 322 µm (30,31) recorded body lengths ranging between 325 and 339 µm depending on the rearing temperature. The width of the dorsal shield in the literature was shorter by 33 - 38µm than that recorded in the present study. Length of 71% of the dorsal setae was shorter and all ventral features under study were longer than those in the Moraes study (31).

Table 1 Effect of laboratory rearing on the morphology of *N. idaeus*. Measurements (mean ± S.E.) in µm of 100 specimens of the feral and colonized strains.

No.	Morph. Feature	Feral			Colonized		
		Mean ± S. E.	Range	CV	Mean ± S.E	Range	CV
1.	DSL	354.5 ±	332 – 397	4.3	352 ± 1.5a	338-378	4.1
2.	DSW	197.8 ± 2b	174 – 242	10.5	186.2 ± 1.2a	167-217	6.5
3.	J1	18.6 ± .2a	16 – 22	9. 9	19.3 ± 0.1b	19-22	6.8
4.	J3	47.3 ± .4b	40 – 53	7.8	44.1 ± .2a	40-47	4.9
5.	J4	42.3 ± .2a	40 – 47	5.5	42.0 ± .2a	40-47	4.0
6.	5	41.6 ± .3a	37 – 47	7.5	43.2 ± 4b	37-50	9.3
7.	J6	52.7 ± 4a	47 – 59	7.1	53. 9 ± . 3b	47-56	5.0
8.	J2	54.7 ± 0.3a	50 – 59	4.7	56. 4 ± 0.3b	53-59	4.6
9.	J5	12. 2 ± 0.1b	9 – 15	9.0	11.6 ± 0.2a	9-16	16.1
10.	Z2	44.2 ± 0.2a	40 – 50	4. 5	44. 5 ± 0.3a	40-56	5.7
11.	Z4	50.9 ± 0.3a	43 – 55	5. 5	53.0 ± 0.3b	49-56	5.0
12.	Z5	34.5 ± 0.2a	31 – 37	6. 3	34.7 ± 0.1a	34-37	3.7
13.	Z1	58.3 ± 0.5b	56 – 81	5.4	56.7 ± 0.1a	56-59	2. 5
14.	Z4	65.8 ± 0.5a	59 – 71	7. 6	70.6 ± 0.3b	62-78	3.9
15.	Z5	72.3 ± 0.3a	65 – 78	4. 2	72.2 ± 0.4a	65-78	5.6
16.	S4	64. 8 ± 0.4a	56 – 71	5.4	64.4 ± 0.3a	59-68	3. 8
17.	S2	62.1 ± 0.2a	49 – 68	6.6	61. 8 ± 0.3a	59-68	4.0
18.	S4	36.9 ± 0.2b	31 – 40	6.6	36. 2 ± 0.2a	34-40	5.3
19.	S5	33. 6 ± 0.3b	31 – 37	8.0	29. 4 ± 0.2a	28-31	5.3
20.	R3	27. 6 ± 0.2a	25 – 31	8. 8	30.3 ± 0.1b	28-31	4. 3
21.	R1	29. 1 ± 0.2a	28 – 31	5.2	30.9 ± 0.3b	28-34	8.7
22.	St IV	46. 5 ± 0.3a	40 – 56	6. 9	47. 5 ± . 2b	43-50	4.6
23.	ST1-ST3	64. 4 ± 0.3b	59 – 71	4.1	62.1 ± 0.3a	59-65	4.3
24.	ST2-ST2	63. 4 ± 0.2b	59 – 68	2.7	62. 0 ± 0.2a	59-65	3. 4
25.	GG	65. 9 ± 0.4b	62 – 74	5. 3	64.8 ± 0.3a	59-68	4.6
26.	VAS-A	89.5 ± 0.4a	68-96	5.0	92. 6 ± 0.3b	87-102	3.2
27.	VAS-P	77. 6 ± 0.6a	68-87	7.7	81. 9 ± 0.2b	81-87	1.9
28.	VAS-L	116.3 ± 0.7b	87-127	6. 0	106. 1 ± 0.8a	87-114	8. 2
29.	Cerv.	13. 3 ± 0.2a	9-15	16.4	13.8 ± 0.2a	12-16	11.2

Means (± S. E.) followed by the same letter in a row are not significantly different ($P > 0.05$).

These differences could be attributed to variations in the population studied by Denmark and Muma (30) and Moraes (31), and the population used in this study (32) (J. S. Yaniniek and A. Onzo, personal communications). Significantly fewer females oviposited in reciprocal crosses between Brazilian and Colombian N.ideaus suggesting that there may be some behavioural isolation affecting copulation (32). First releases of the Brazilian biotype in the Republic of Benin and Kenya to control CGM have been recovered in follow up surveys for longer periods than release durations of the Colombial biotype (33).

Table 2 Effect of laboratory rearing on the ratio of closely related morphological features of *N. idaesus*.

No	Morph Group	Feral Mean ± S.E.	CV	Colonized Mean ± S.E	CV
1.	J4/j5	1.02 ± 0.01a	9.43	0.98 ± 0.01b	9.57
2.	Z5/j5	5.99 ± 0. 70b	11.99	6.40 ± 011a	16.74
3.	r3/R1	0.95 ± 0.01b	9.71	0.99 ± 0.01a	9.48
4	Z1/S4	1.59 ± 0. 01a	8.48	1.57 ± 0.01a	5.59
5.	DSL/DSW	1.81 ± 0. 02b	10.39	1.90 ± 0. 01a	7.15
6.	ST1-ST3/ ST2-ST2	1.02 ± 0.005a	4.74	1.01 ± 0.01a	5.75
7.	VAS-A/VAS-L	0.77 ± 0.01b	7.60	0.88 ± 0.01a	8.94

Means (± S. E.) followed by the letter in a row are not significantly different (P > 0.05). N = 100

The increase in length recorded for 42% of the dorsal setae, namely j1, j5, j6, j2, z4 and Z4 and macroseta on leg IV in the colonized strain may indicate an adaptation for feeding on prey mite with dense webbings. The long setae like j5, j6, j2, and z4 and Z4 are used by the predator to penetrate dense webbing of tetranychids during search for food. A direct reationship between incidence of long dorsal setae in phytoseiids preying on phylophagous mites with dense webbings has been suggested. Phytoseiid predators were thought to penetrate the webbing of the prey by using their long dorsal setae as a wedge (34). The less specialized *L. degenerans* with minute dorsal setae (35) consistently avoided leaf surfaces covered with silken threads of *T. pacificus* while the more specialized *P. persimilis* with long slender dorsal setae (35) distributed itself on silk-covered leaves (36). Schmidt (37) reported that the webs of *Tetranychus* species attracted *P. persimilis* more than prey eggs or exuviae *M. occidentalis* whose dorsal setae are moderately long (35) exhibited decreased walking speed, increased turning rate and entered a specialized search mode upon encountering certain spider mite silken webs and in this state, the chance of locating a prey was increased (38). The colonized strain has been mass produced on *T. urticiae*, a species that produces dense webbings (39) although it was meant for the classical biological control of CGM, a species that produces little or no webbing (33,40). In the rearing cultures, eggs supplied as food often hatch into larvae that produce dense webbing which eventually acts as a barrier to the remaining eggs. Another feature in the rearing unit that can hinder the movement of predators is the cottonwool oviposition site. To overcome these searching barriers, colonized *N. idaeus* developed longer dorsal setae which it uses as a wedge. Dorsal shield setae that showed no significant changes between both strains were characteristically long (57 ± 12 µm) with respect to others in similar relative positions with the possible exception of z5. Also, the ratios of closely related diagnostic characters stongly suggest enlongation of these features in the colonized strain.

The smaller ranges recorded for 75% and lower coefficient of variation (%) recorded for 69% of the colonized strain's features suggested more diversity in the feral population. It is usual to start a laboratory culture with population that is less representative of the naturally occurring population (41,42). This could be the reason why a narrower range representing a subset of the natural population of *N.idaeus* was observed in the colonized strain. On the other hand, laboratory selection pressure could have reduced the

population diversity leading to further convergence of external morphological features resulting in smaller genetic variability (15). Consequently, wherever possible, it is recommended that the predator be reared on its original primary prey and the original host plant under near natural conditions to limit morphological changes in the closed laboratory population.

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