Intraspecific Variations of Idiosomal Setal Patterns of Phytoseiid Mites

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ABSTRACT

Intraspecific variation of idiosomal setal patterns was examined in field populations of eight phytoseiid species and in laboratory populations of Neoseiulus californicus and N. womersleyi. In field populations, about 6.4–16.1% of females showed setal variation, whereas in laboratory populations, this percentage was relatively low in N. californicus (5.8–14.8%) but high in N. womersleyi (8.6–36.1%). Setal variations were divided into the following categories: absent, additional, inserted, deviated, expanded, and shortened setae. An absence of setae was more common than additional or inserted setae, both in the field and laboratory populations. Expanded and shortened setae were rare. An absence of setae was frequently observed on the ventral opisthosoma, and occurred on both of its sides (left and right), mainly at ZV3 in N. womersleyi and JV3 in Typhlodromus vulgaris. In the IG population of N. womersleyi, an absence of setae was frequently observed at ZV1 (49.5% of the total absent setae in the population) and ZV3 (37.8%), which was significantly different from the other populations. The difference in setal variation between field and laboratory populations suggests that it is not caused by accidental defects during post-embryonic development but rather is the result of heritable traits. The maintenance of the absence of setae in field populations may create a driving force from holotrichous to hypotrichous conditions, and may produce higher levels of species diversity in phytoseiid mites.

Key words: phytoseiid mites, setal pattern, intraspecific variation, hypotrichous condition, paedomorphosis

INTRODUCTION

The family Phytoseiidae is a large group in the order Mesostigmata; it contains 15% of the known diversity in the order (Krantz and Walter, 2009). The number of nominal species in Phytoseiidae was 2,280 in 2007 (Chant and McMurtry, 2007) and it has been gradually increasing (e.g. Khan et al., 2011). One of the taxonomic characters used in the description of new species of phytoseiid mites is the idiosomal chaetotaxy, the pattern and length of setae on the dorsal and ventral body. Phylogenetic relationships within the family Phytoseiidae and among closely related families have also been investigated using this character (Chant and Yoshida-Shaul, 1992; Chant, 1993a). The number of idiosomal setae on phytoseiid mites ranges from 25 to 38 pairs.

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(left and right setal pairs). Interspecific variation in idiosomal setal patterns has been divided into 85 patterns in adult females (Chant and Yoshida-Shaul, 1992), and the high diversity in setal patterns was recognized as a marked morphocline from the ancestral state (plesiomorphic holotrichous condition) to descendent species (apomorphic hypotrichous condition) (Chant, 1993a).

According to a numerical analysis of setal patterns of phytoseiid females, 99 single transformations have been recognized among the 85 setal patterns (Chant, 1993a). These transformations also support an evolutionary process from holotrichous towards hypotrichous conditions. The hypotrichous condition of adult females is expressed as if the relatively low number of setae that are present during immature stages is maintained even after emergence. Thus, it is presumed that paedomorphosis is a driving force for the transformation from ancestral to descendent states (Chant, 1993b).

However, the 85 setal patterns cannot be explained solely by paedomorphosis but must involve other factors, because the diversity in setal patterns is extremely uneven across the family. The distribution of setal patterns is quite different among the three major groups Amblyseiinae, Phytoseiini, and Typhlodromini (the latter two are classified, at present, as subfamilies of Phytoseiinae and Typhlodrominae) (Chant, 1993a). In addition, the setal patterns converge on a few forms: one major pattern is found in 49% of phytoseiid species (*Amblyseius obtusus* type) and a second speciose pattern (*Typhlodromus rhenanus* type) is found in 14% of the species (Chant and Yoshida-Shaul, 1992). It is also estimated that the major pattern is a complex of different clades, each of which was derived from six precursors and gave rise to seven derivative patterns. Similarly, the second pattern may also be a complex of different clades, each of which was derived from two precursors and gave rise to three derivative patterns (Chant, 1993a).

In this paper, intraspecific variation in setal patterns was examined to obtain collateral evidence of dynamic evolution of phytoseiid diversity. Because the family Phytoseiidae shows wide morphological diversity and the number of species is still gradually expanding, intraspecific variation in setae may provide evidence of ongoing speciation in this group. In fact, a pair of dorsal setae, j1, was unstable in a laboratory colony of *Phytoseiulus longipes*: 42% of the specimens had only one of the pair and 16% had lost the whole pair (Takahashi and Chant, 1993). Although it is very rare, we can find similar setal variation in specimens collected in the field. Because discontinuous variables can easily be identified without taxonomic skills and experience, intraspecific variation in phytoseiid specimens collected in the field and from laboratory cultures was used to quantify and trace adaptive radiations from the major patterns observed in phytoseiid species. Eight species that were collected in sufficient numbers from the field were examined to estimate the frequencies of variation in the field. Thirteen laboratory populations of two species were used to evaluate the possibility that certain setal traits evolved and became fixed due solely to laboratory rearing conditions.

**MATERIALS AND METHODS**

Specimens of eight species were collected from apple orchards, their surrounding natural habitats (*lat. 39°45’N; long. 141°08’E*), and tea plantations (*lat. 34°47’N; long. 138°08’E*) (Table
1). All specimens were mounted in Hoyer’s medium and observed under a microscope using phase contrast and differential interference contrast devices (Olympus BX53). All specimens except individuals of *Typhlodromus vulgaris* showed the *A. obtusus* type of setation; *T. vulgaris* showed *T. rhenanus* type setation (Fig. 1). Females with an egg in their body were excluded from the study because several setae were invisible because of the opaque egg. Much variation was detected during preliminary observations, and the setae were classified as absent, additional, inserted, deviated, expanded, or shortened setae. Absent setae were used to describe those that did not grow from the original position without a setal pore (Fig. 2a). Setal pores without setae were not counted as the absent setae because those pores had a possibility to lose the setae during handling and mounting procedures. Additional setae were those that grew together at the same position (Fig. 2b). Inserted setae were those that grew at a potential position for either the *A. obtusus* or *T. rhenanus* types (Fig. 2c). Deviated setae were those that had moved from the original position, especially inside or outside of the plates (Fig. 2d). Expanded and shortened setae were also identified when the width and length of the setae were conspicuously different from those at the same position on different individuals (Fig. 2e,f).

Four populations of *Neoseiulus californicus* and nine populations of *N. womersleyi* were also examined in the same manner. Populations were sampled at different sites in Japan. Collection records and abbreviated name of each population referred to Toyoshima and Hinomoto (2004) for *N. californicus* and Toyoshima and Hinomoto (2003) for *N. womersleyi*. These populations had been maintained with *Tetranychus urticae* as prey on bean leaflets for more than 10 years in the laboratory. Over a period of more than 10 weeks, approximately 20 females were collected each week from each rearing culture and mounted in Hoyer’s medium.

### Table 1. Collection periods and sites of phytoseiid specimens for setal investigation

<table>
<thead>
<tr>
<th>Species</th>
<th>No. females tested</th>
<th>Collection period</th>
<th>Collection site</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Neoseiulus californicus</em></td>
<td>490</td>
<td>2007/6/19 ~ 2009/11/16</td>
<td>Apple orchards</td>
</tr>
<tr>
<td><em>Amblyseius orientalis</em></td>
<td>374</td>
<td>2006/5/8 ~ 2009/10/5</td>
<td>Natural habitat</td>
</tr>
<tr>
<td><em>Amblyseius kokufuensis</em></td>
<td>188</td>
<td>2006/6/19 ~ 2008/10/21</td>
<td>Natural habitat</td>
</tr>
<tr>
<td><em>Amblyseius eharai</em></td>
<td>1092</td>
<td>2009/5/15 ~ 2009/7/13</td>
<td>Tea plantation</td>
</tr>
<tr>
<td><em>Euseius finlandicus</em></td>
<td>563</td>
<td>2006/5/15 ~ 2009/11/4</td>
<td>Natural habitat</td>
</tr>
<tr>
<td><em>Euseius sojaensis</em></td>
<td>613</td>
<td>2009/5/15 ~ 2009/6/23</td>
<td>Tea plantation</td>
</tr>
<tr>
<td><em>Typhlodromus vulgaris</em></td>
<td>3258</td>
<td>2007/5/11 ~ 2009/11/11</td>
<td>Apple orchards &amp; Natural habitat</td>
</tr>
</tbody>
</table>

1) Main site is shown for all but *T. vulgaris*.

“Natural habitat” is located around “Apple orchards” (lat. 39°45′N; long. 141°08′E).

“Tea plantation” is located at lat. 34°47′N and long. 138°08′E.
RESULTS AND DISCUSSION

Setal variation in the field

In the field, 6.4–16.1% of the females of each phytoseiid species showed setal variation (Fig. 3). The number of females with setal variation correlated well with the number of females examined; this relationship can be estimated by the equation \( y = 0.0966x + 6.7163 \), where \( y \) is the number of females with setal variation, and \( x \) is the number of females investigated (\( R^2 = 0.86 \)). This equation estimates that ca 10% of females in the natural and agricultural habitats had setal variation. Among the total variation, 4.2%, 8.9%, 23.3%, and 63.1% of females had inserted, additional, deviated, and/or absent setae, respectively. In addition, there were three cases of expanded and one case of shortened setae.

Although most females showed variation in a single setal trait, some females had multiple types of setae (Fig. 4). For example, some had two absent setae on the left and right symmetrical positions (hereafter, paired variation). Based on the random combination of two setae among the dorsal and ventral setae, the possibility of paired variation is ca 1.5%; hence, of the 2,080 setal combinations of the *Amblyseius obtusus* type and 2,346 setal combinations of the *Typhlodromus rhenanus* type, paired variation could have occurred in a total of 32 and 34 cases, respectively. Because 40 female *N.*
Fig. 2. Types of setal variants in phytoseiid females. (a) Absent seta. Setae and setal pores are absent at the appropriate position. (b) Additional seta. Two setae come up together at the original position. (c) Inserted seta. A single seta comes up at a potential position for *A. obtusus* or *T. rhenanus* types. (d) Deviated seta. The setae have moved from their original positions, especially from inside to outside of the plates, or vice versa. (e) Expanded seta. A single seta is more expanded than an original seta at the same position. (f) Shortened seta. A single seta is shorter than an original seta at the same position.
*womersleyi* had absent setae at the two positions, if the two absent setae were combined randomly a total of 0.6 females would be expected to show paired variation. However, more females than expected had paired variation in both *N. womersleyi* and *T. vulgaris* (Table 2). A linear regression of paired variation against cases of multiple setal types (hereafter, multiple variation) \( (R^2=0.93) \) showed that 11.4% of cases with absent setae at the two positions were combined as paired variation. This means that paired variation more easily occurred at certain

Fig. 3. Proportion of phytoseiid females with setal variant in field populations. Each female has a single variant or multiple variants of absent, additional, or deviated setae.

Fig. 4. Proportion of phytoseiid females with a single setal variant and multiple variants.
positions (see Table 2). This tendency may be a driving force in the evolution of new species of phytoseiid mites. When the *A. obtusus* type loses a pair of setae at ZV3 or JV4, the setal pattern becomes the same as that of the *Amblyseius berlesei* type or the *Amblyseius havu* type, respectively, according to Chant and Yoshida-Shaul (1992). Based solely on the viewpoint of variation in setal pattern, *N. californicus* or *N. womersleyi* are ancestral candidates for phytoseiid species that have the *A. havu* type setal pattern, and *N. womersleyi* or *Euseius finlandicus* are ancestral candidates for *A. berlesei* type species. Paired variations of j1, ST4, and ZV1 from *A. obtusus* type individuals do not create candidates for any currently known phytoseiid species. Similarly, paired variations in *T. vulgaris* do not produce possible candidates for any current phytoseiid species.

Subsequently, we focused on absent setae for a more detailed analysis. Absent setae occurred mainly at setal positions on the ventral surface (Fig. 5). In five species, absent setae were strongly biased onto the ventral opisthosoma. In *N. womersleyi*, absent setae were frequently observed at JV4 (16.5% of the total deletions in *N. womersleyi*), ZV1 (20.0%), and ZV3 (30.9%). The tendency for absent setae in *N. womersleyi* may have resulted in the paired absent setae depicted in Table 2. Absence at ST4 was also frequently observed (10.4%) and can lead to paired variation, although species with this type of variation have not yet been described in phytoseiid mites. The absence of setae at ST4 (25.0% of the total deletions in *N. californicus*) and JV4 (14.7%) can lead to paired variation in *N. californicus*. In contrast, in *Euseius sojaensis*, the absence of setae at ZV1 (30.0% of the total deletions in *E. sojaensis*) may lead to speciation from the *A. obtusus* type to an unknown setal pattern type, although paired variation was not observed.

*T. vulgaris* was the only species that frequently showed the *T. rhenanus* type in the field. The tendency for setal variation was similar to that of *A. obtusus* type species: in the field, almost 10% of females had setal variation (Fig. 3), with a change in a single trait being the most common (Fig. 4) and distributed over the ventral opisthosoma (Fig. 5). The most common type of variation was absent setae. Paired absent setae were frequently observed at JV3 (Table 2). Based solely on the viewpoint of variation in setal pattern, females with paired absent setae at JV3 may be misidentified as a species of the *Typhlodromus singularis* type (Chant and Yoshida-Shaul, 1992).

<table>
<thead>
<tr>
<th></th>
<th>No. total variations</th>
<th>Absent j1</th>
<th>ST4</th>
<th>JV2</th>
<th>JV3</th>
<th>JV4</th>
<th>ZV1</th>
<th>ZV3</th>
<th>Additional JV1</th>
<th>Deviated r3</th>
<th>JV1</th>
<th>ZV2</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>N. californicus</em></td>
<td>99</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>N. womersleyi</em></td>
<td>302</td>
<td>2</td>
<td>3</td>
<td>1</td>
<td>1</td>
<td>7</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>A. eharai</em></td>
<td>81</td>
<td>2</td>
<td>3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>E. finlandicus</em></td>
<td>43</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>E. sojaensis</em></td>
<td>78</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>T. vulgaris</em></td>
<td>343</td>
<td>1</td>
<td>1</td>
<td>5</td>
<td>2</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
<td>2</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*A. orientalis* and *A. kokufuensis* do not have any paired variations. The paired setal variation in this table is defined as that the left and right setal pairs show the same variation together.
Setal variation in laboratory populations

Setal variation was also variable in the laboratory populations of *N. californicus* and *N. womersleyi*. Of these populations, 5.8–14.8% of female *N. californicus* and 8.6–36.1% of female *N. womersleyi* (Fig. 6) showed setal variation. Because the initial rates of variation were not recorded, it is not known whether they changed during long-term rearing in the laboratory. However, according to differences in the rates of setal variation among laboratory populations that were maintained under identical rearing conditions, it is plausible that the setal variation stemmed from variation in heritable traits rather than accidental defects during post-embryonic development. The accumulation of phenotypic variation in laboratory cultures depends on the local population itself or is skewed by the sample size at the start of rearing for each laboratory population. Phenotypic variation will become fixed by chance if there is no trade-off in fitness between individuals with and without setae.

The rates of absent, additional, and deviated setae were 4.4–12.4%, 0.0–1.4%, and 0.0–4.3% in *N. californicus*, and 4.2–34.7%, 0.0–4.8%, and 0.5–8.6% in *N. womersleyi*. This pattern is similar to what was observed in the field populations. Although the rate of variation in three populations of *N. californicus* was similar to the field collections (16.1%), the variation in SP was smaller among the populations. Additional setae were not found in the SP population, but absent and deviated setae were observed in 10 and 3 females, respectively. In particular, ST3 was absent in 16 females in the MK populations.

In *N. womersleyi*, variation was common in the IG population. Of 565 females that were examined, 171 females had 196 absent, 2 additional, and 6 deviated setae. Of the 196 absent
setae, 130, 54, and 12 absent setae were observed as single, double, and triple variants, in which a female simultaneously lost 1, 2, or 3 setae, respectively. The 27 combinations of two absent setae were ZV1 & ZV3 (14 cases), ZV1 & ZV1 (4), ZV3 & ZV3 (3), JV4 & ZV3 (2), ZV2 & ZV2, r3 & ZV3, JV2 & ZV3, and J2 & ZV1. The four combinations of three absent setae were ZV1 & ZV1 & ZV3 (2 cases), JV2 & ZV1 & ZV3, and JV4 & ZV3 & ZV3. Except for the combination of ZV2 & ZV3, ZV1 and ZV3 were combined with ZV3, ZV1, or other setae. In fact, absences of ZV1 (49.5% of the total absences in the IG population) and ZV3 (37.8%) were frequently observed in the IG population, which was similar to the field collections of *N. womersleyi*, despite their different origins. The IG population was originally collected on a small island in southern Japan in 1996 (Toyoshima and Hinomoto, 2003), but the field collection of *N. womersleyi* that is shown in Figs 3–5 was derived from northern Japan (Table 1). This may indicate that ZV1 and ZV3 easily become absent in *N. womersleyi*. Although it was very rare, paired variations were also observed in two cases with three absent setae and four cases with two absent setae at ZV1, and in one and three cases at ZV3, respectively, as mentioned above, in the IG population.

ZV1 and ZV3 are categorized as variable setae in the phylogeny of the family Phytoseiidae (Chant and Yoshida-Shaul, 1992). There is also considerable variation in the presence/absence of both setae in the genus *Neoseiulus* (Chant and McMurtry, 2003), which is the common genus of the *Amblyseius obtusus* type. For example, ZV1 is absent in *N. mumai* Denmark and ZV3 is absent in *N. leucophaeus* (Athias-Henriot) and *N. reductus* (Wainstein). ZV3 is also absent in *Pholaseius colliculatus* Beard, which has a reduced ventrianal plate (Beard, 2001). Thus, females without ZV1 or ZV3 pairs would be recognized as a new species if the setal variation in the IG population were genetically fixed in the field. On the other hand, ZV1 and ZV3 should be recognized as ontogenetically variable setae. In the ontogeny of the *Amblyseius obtusus* type, the setae ZV1 and ZV3 are not seen in the larval and protonymphal stages; females gain them during the molt from the protonymphal to the deutonymphal stage, whereas males do not gain these during this molt (Rowell and Chant, 1978). The absence of ZV1 and ZV3 in the IG population may be caused by mutations in genes that are expressed during this molt, which may be evidence of a driving force in *N. womersleyi* towards a hypotrichous condition in relation to paedomorphosis. Among the other populations, the absence of ZV1 was more frequent even in the AS (32% of the total deletions in the strain) and SI (25%) populations, and ZV3 was frequently absent in the KI (53%), AS (26%), and SI (16%) populations. As an exception, absent setae were observed at j4 (30%) and JV4 (24%) in the CH population. However, the results of this study are not sufficient to explain the relationship between phylogenetic variation in setal suppression and setal development during ontogeny.

**Conclusion**

Intraspecific variation in idiosomal setation was maintained at similar proportions in field populations, but differed among laboratory populations even though they were reared under identical conditions. Thus, it is assumed that setal variation is not the result of ontogenetic defects but rather is due to variation in heritable (phenotypic) traits, although the absence of setae on the ventral opisthosoma (ZV1, ZV3, JV3, and JV4) matched the patterns that would result if
suppression during post-embryonic development was maintained.

The tendency to maintain absent setae more than additional and inserted setae was comparable between the field and laboratory populations. This tendency in each species may be the result of an evolutionary process from a holotrichous toward a hypotrichous condition in phytoseiid mites, although the absence of setae is not necessary for speciation in this group. In fact, N. womersleyi was discriminated from N. longispinosus by a difference in the length of L8 (S5, present nomenclature) alone on the dorsal shield (Schicha, 1975), and the reproductive isolation between two species was confirmed (Ho et al., 1995). We assume that phytoseiid species always experience a driving force to change their genetic elements to adapt to new habitats. It seems that this driving force primarily produces and maintains much of the diversity in phytoseiid mites, and that the setal variation detected in this study was a byproduct of this diversification. Although speculation about genetic elements related to setal variants and their inheritance is beyond the scope of this study, we assume that future studies will find several new species that will fill gaps in the discontinuity of idiosomal chaetotaxy between ancestral and descendent conditions.

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REFERENCES


摘要
カブリダニの胴毛パターンの種内変異
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カブリダニの胴毛パターンの種内変異を野外個体群および室内飼育個体群で調査した。8種の野外個体群では6.4〜16.1%、ミヤコカブリダニの飼育個体群では5.8〜14.8%、ケナガカブリダニの飼育個体群では8.6〜31.6%の雌成虫が胴毛の変異を示した。これらの雌毛の変異は、欠失、追加、挿入、移動、幅広、短小に類別され、欠失が追加や挿入よりも多く、幅広や短小は希であった。欠失は後胴体部の腹面に多く見られ、野外個体群のケナガカブリダニではZV3、フツウカブリダニではJV3で左右同時に欠失が観察された。ケナガカブリダニのIG飼育個体群では、ZV1（個体群内で観察された全欠失の49.5%）とZV3（同37.8%）で多く観察された。この類度は他の飼育個体群に比べて突出して多かった。野外個体群に比べて室内飼育個体群の変異幅が広いことから、これらの胴毛の変異は発生過程における偶発的な事象というよりも遺伝形質であることが示唆された。胴毛の欠失に関する遺伝
要素が野生個体群に維持されるのは、系統発生で仮定されている多毛から貧毛への進化的方向性を支持する可能性がある。また、胴毛欠失が遺伝的に固定されやすいことは、カブリダニの種多様性の向上に寄与する可能性を示す。