

THE MISSING STASE IN SPIDER MITES (ACARI: TETRANYCHIDAE): WHEN THE ADULT IS NOT THE IMAGO

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ABSTRACT — Apart from a calyptostatic prelarva, the ontogeny of spider mites is limited to one six-legged immature followed by three eight-legged instars. The usage consists in naming them: larva, protonymph, deutonymph and adult. In this study, the "missing stase" and different associated hypotheses are explored using diverse approaches: light microscopy, electron microscopy, comparative chaetotaxy, ontogenetic trajectories, and interspecific comparisons. It turns out that the imago (new name for the last stase) is missing in *Tetranychus urticae* and that its post-embryonic development may be summarized as follows: prelarva (calyptostase), larva (six-legged stase), protonymph (eight-legged stase), deutonymph (eight-legged stase), tritonymph (with paedogenesis, *i.e.* precocious development of sexual maturity). The respiratory role of the prelarva is emphasized. The current terminology of post-embryonic development is confusing as it pertains to three approaches: the state or form description, the instar standard and the stase paradigm.

KEYWORDS — *Tetranychus urticae*; ontogeny; ontogenetic trajectory; paedogenesis; stase; state; instar; respiration

INTRODUCTION

Aside from ticks, Tetranychidae are the most studied mites; a huge amount of literature is devoted to their biology (see Helle and Sabelis, 1985) and they have been proposed as a chelicerate model organism (Grbić *et al.*, 2007). The ontogeny of spider mites is noteworthy in that it comprises only four active stases. Surprisingly, no attempt has been made to elucidate the true identity of these active stases or to demonstrate that the so-called "missing" stase was really missing or merely calyptostatic.

Apart from a calyptostatic prelarva described by Grandjean (1948), the ontogeny of spider mites includes one six-legged immature followed by three

eight-legged instars. The usage consists in naming them: larva, protonymph, deutonymph and adult. These names imply that the "missing stase" is the tritonymph. Yet, any stase is likely to be "missing" or, at least, to be calyptostatic. These names also imply that the first eight-legged instar is homologous to the protonymph of other actinotrichid mites with five active stases. This has not been demonstrated and it is not by chance that Grandjean (1948) used the expressions larva, first and second nymphs instead of the prevailing appellations. Let's designate this usual interpretation, the null hypothesis, H_0 (Fig. 1B).

A priori, three alternative hypotheses may be advanced. The first hypothesis, H_m , is a multiple

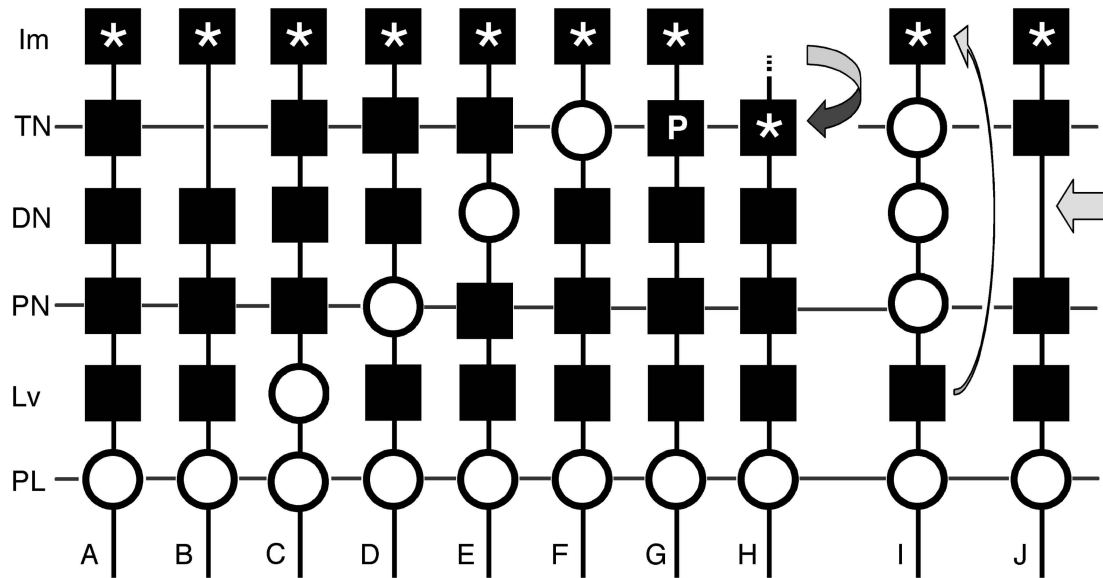


FIGURE 1: Ontogeny in Tetranychoida (A – H) and other mites (I – J). A – Plesiomorphic ontogeny observed in Tuckerellidae; B – Tetranychidae (traditional interpretation – hypothesis H_0); C – multiple protelattosis in Tetranychidae (hypothesis H_m); D-E-F – metelattosis in Tetranychidae (intervening calyptostasis, hypothesis H_i); G – Tuckerellidae with a paedomorphic tritonymph; H – progenesis in Tetranychidae (hypothesis H_p); I – three calyptostasic nymphs in Ereynetidae (Speleognathinae); J – only four active stases in Astigmata (Psoroptidae). Im: imago; TN: tritonymph; DN: deutonymph; PN: protonymph; Lv: larva; PL: prelarva. Close square: active homostase; Open circle: calyptostase; P: paedomorphic tritonymph; *: reproductive system.

protelattosis (Fig. 1C). Protelattosis, *i.e.* the occurrence of a calyptostase at the beginning of ontogeny (Hammen, 1975, 1980), is widespread in Arachnida (André and Jocqué, 1986). In Tetranychidae, as in most Actinotrichida, the prelarva is calyptostatic and remains within the egg (Grandjean, 1948). Multiple protelattosis involves the succession of several calyptostases at the beginning of the ontogeny. In Tetranychidae, this hypothesis implies that both the prelarva and the larva would be calyptostatic as already hypothesized in Myobiidae (Grandjean, 1938a). Some larvae tend to be weak, sluggish, nonfeeding forms (Walter and Krantz, 2009). As a result, the first active stage would be a six-legged protonymph. Strictly speaking, the presence of only six legs in a stage does not suffice to identify a larva and Grandjean (1938b: 1349) already deplored that acarologists were used to designate as larva any stage with six legs.

The second hypothesis, H_i , is metelattosis *sensu* Hammen (1975, 1980), which supposes the presence of a calyptostase intervening between two active stases in the course of ontogeny (Fig. 1D,E,F). Such

calyptostases are detectable through the presence of an apoderm or other reduced organs. If the calyptostase is demonstrated to be the tritonymph (Fig. 1F), then H_i is similar to H_0 as far as active stases are concerned; a major difference remains, however, that in the latter case, the tritonymph is really missing while, in the former, it is calyptostatic.

A third and last hypothesis, H_p , presupposes that the "missing stage" lies at the other end of the ontogeny and, in the absence of imago, implies that reproduction is taken over by the tritonymph, *i.e.* progenesis or paedogenesis (Fig. 1H).

To improve our understanding of the ontogeny of spider mites, we thus need to solve two problems. The first problem involves the identification of active stases. The second consists in checking whether the "missing stage" is really missing (meristasy) or reduced to a calyptostase (heterostasy). The first question may be addressed through comparative chaetotaxy and ontogenetic patterns. The second question, the detection of a calyptostase occurring somewhere in the ontogeny of spider mites, requires careful examination of pharate mites or the

use of electron microscopy techniques as suggested by André (1992).

MATERIALS AND METHODS

Species

Species used in comparative tests belong all to Actinotrichida (Table 1). Observations on *Tetranychus urticae* are also based on spider mites obtained from the permanent rearing of the ecology laboratory.

Terminology

There are four active stases in Tetranychidae. We number them from S_1 to S_4 . Tuckerellidae have five active stases that may also be numbered, from A_1 to A_5 . In contrast to Tetranychidae, an ontogenetic level may be assigned to tuckerellid stases, from A_1 corresponding to the larva to A_5 corresponding to the imago.

Apolysis is taken as the criterion to recognize the occurrence of a new stase as discussed by Hinton (1971, 1973, 1976), Coineau (1974) and Fink (1983). Pharate animals are thus designated by the name (or number) of the inner stase.

Observation of the egg

Due to the difficulties in preparing mite eggs for TEM studies, we did not reiterate embryological studies by Dittrich (1968, 1971) and Dittrich and Streibert (1969). All their observations were made at 24 °C. As Dittrich was unaware of Grandjean's

work (Dittrich, *in litteris*), we briefly re-interpret his observations at the light of the stase concept.

Observation of pharate mites

Pharate mites were prepared for transmission electron microscope (TEM) studies. *Tetranychus urticae* were fixed in glutaraldehyde and treated as in André and Remacle (1984).

Comparative chaetotaxy

Chaetotaxy of spider mites and its development have been studied by Wainstein (1958), Robaux and Gutierrez (1973) and Lindquist (1985). As ontogeny of chaetotaxy in Tuckerellidae, a tetranychoid family with five active stases, was described by Quiros-Gonzalez and Baker (1984), it is possible to carry out a comparative study of chaetotaxy of spider mites.

As homologies of leg chaetotaxy have not been fully determined on a comparative manner between Tetranychoida (Lindquist, 1985), a quantitative approach was favored. For instance, the gain in setae on each leg segment may be compared between homologous stases of different mite families. Another quantitative approach is offered by the study of ontogenetic trajectories as explained hereafter.

Ontogenetic trajectories

The ontogenetic trajectory method (André, 1988) involves plotting points representing the stases in an n -dimensional character space and connecting the points representing the successive stases of a species. In this case, the stases were plotted in a

TABLE 1: Species used in quantitative analyses (Fig. 4).

Species	Classification	Reference
<i>Terpnacarus bouvieri</i>	Endeostigmata Terpnacaroidea Terpnacaridae	Grandjean (1939)
<i>Edbakerella marshalli</i>	Prostigmata Tydeoidea Triophtydeidae	André (1980)
<i>Pretriophtydeus tilbrooki</i>	Prostigmata Tydeoidea Triophtydeidae	André (1980)
<i>Tydeus spathulatus</i>	Prostigmata Tydeoidea Tydeidae	André (1980, 2005)
<i>Pronematus ubiquitus</i>	Prostigmata Tydeoidea Iolinidae	André (1980)
<i>Iolina nana</i>	Prostigmata Tydeoidea Iolinidae	André (1984)
<i>Tuckerella ornata</i>	Prostigmata Tetranychoida Tuckerellidae	Quiros-Gonzalez & Baker (1984)
<i>Tetranychus urticae</i>	Prostigmata Tetranychoida Tetranychidae	Vainshtein (1958), Robaux and Gutierrez (1973), Lindquist (1985)

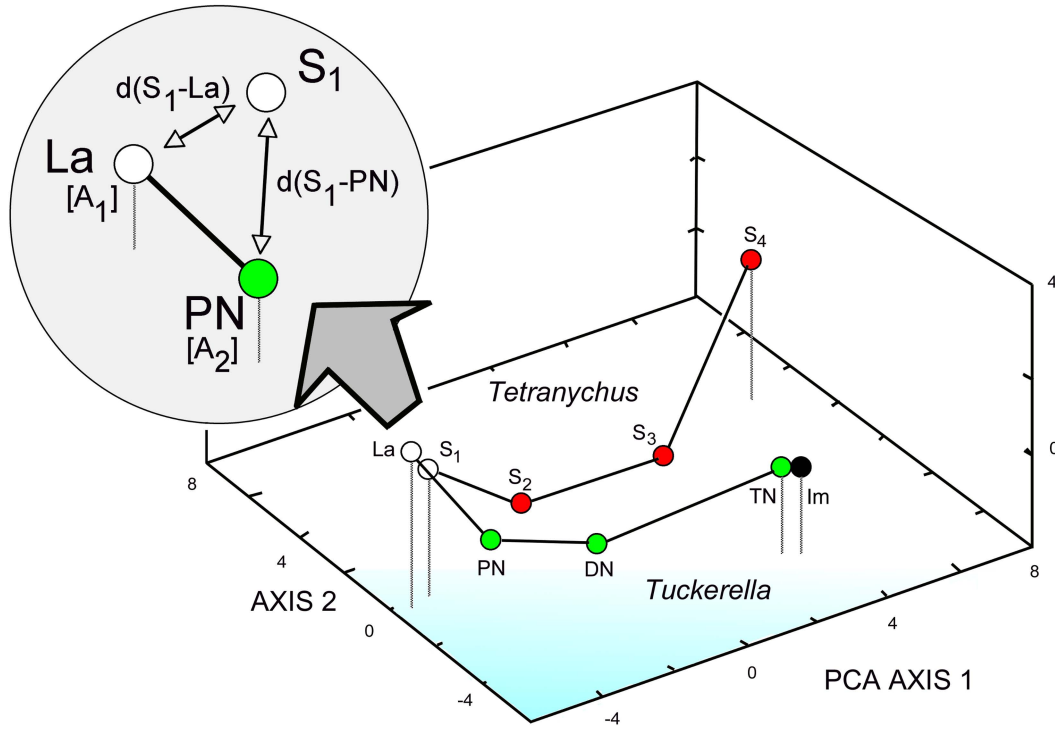


FIGURE 2: Ontogenetic trajectories of Tetranychidae and Tuckerellidae. Trajectories were plotted in a 27-dimensional space corresponding to chaetotaxy of leg segments, epimeral and genital chaetotaxy and, subsequently, projected into a 3-dimensional space through PCA. The insert describes the way distances are measured between homologous stases depending on the hypothesis chosen (H_m vs H_p).

27-dimensional space, corresponding to the chaetotaxy of the 20 leg segments plus four axes describing the epimeral chaetotaxy and three for the genital formula (eugenital, aggenital, genital setae). Principal component analysis (PCA) was used to reduce these dimensions and project the 27-dimensional trajectories into a space of three dimensions. As the characters used are supposed to be discrete and independent, distances were estimated through the Manhattan metric (rather than Euclidian distance or Chebyshev distance) as made previously (e.g. André and Fain, 1991).

Distances may be measured in the character space between homologous stases belonging to different ontogenetic trajectories. Two sets of distances were measured corresponding to two different hypotheses. Either the first active stage of Tetranychidae, S_1 , is supposed to be a larva as under the pae-

dogenet hypothesis, H_p ; then the distance is measured between homologous stases, i , along all dimensions, j :

$$d(S_i-A_i) = \sum_{j=1-27} |S_i-A_i| \text{ [see Fig. 2]}$$

The mean distance between homologous stases is

$$d = 1/4 \sum_{i=1-4} d(S_i-A_i)$$

Otherwise, if the first stage of Tetranychidae is homologized with a protonymph, *i.e.* with the second stage of Tuckerellidae as under the multiple protellatosis hypothesis, H_m , then:

$$d(S_i-A_{i+1}) = \sum_{j=1-27} |S_i-A_{i+1}| \text{ [see Fig. 2]}$$

The mean distance between homologous stases is computed similarly.

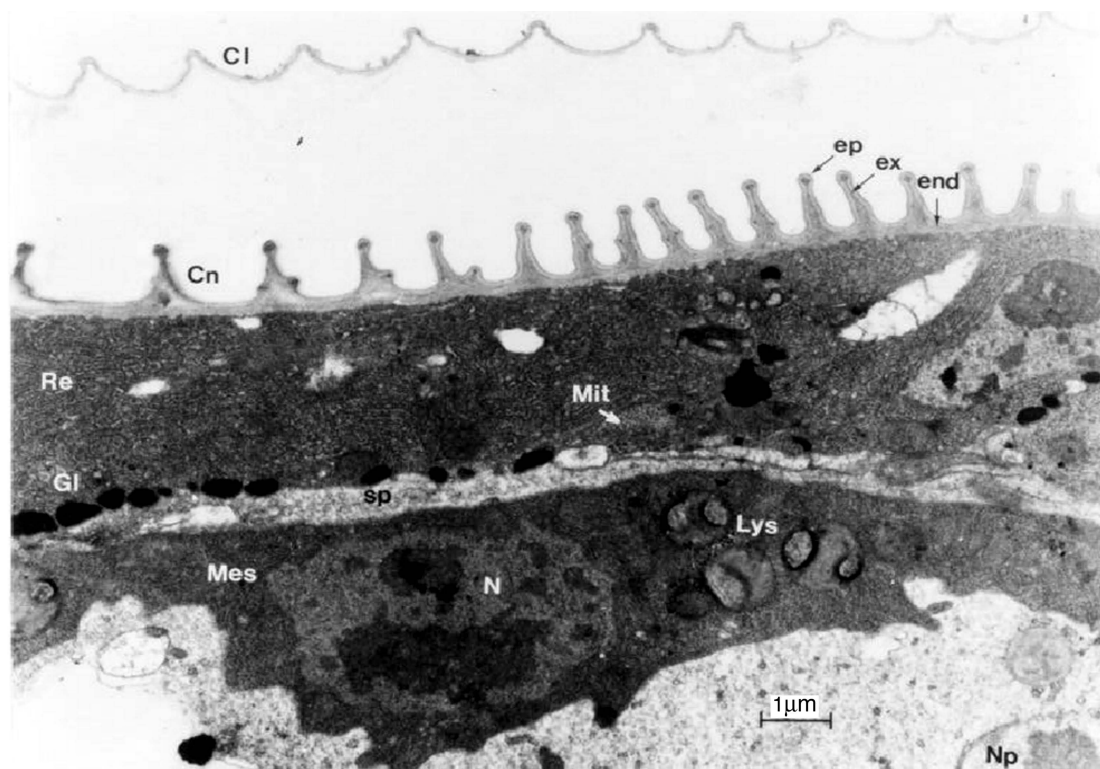


FIGURE 3: *Tetranychus urticae*, TEM of a pharate S1 transforming into a S2. Abbreviations: Cl : S1 cuticle; Cn: S2 cuticle; en: endocuticle; epi: epicuticle; ex: exocuticle; Gl: droplet of a not saturated lipid; Lys: lysosome; Mes: mesenchyme; Np: pycnotic nucleus of a lysed cell; Mit: mitochondria; N: nucleus; Re: endoplasmic reticulum; sp : intercell space (from Van Impe, 1985).

The length, l , of any segment of ontogenetic trajectories, *i.e.* the distance between two successive stases, may be estimated in the same way. The sum of all segments constituting a trajectory gives its total length, L . The l/L ratio estimates the contribution of a stase to the trajectory.

RESULTS

Observation of the egg

According to Dittrich's publications, the intermediate lamella, formerly named intermediate membrane (Dittrich and Streibert, 1969), appeared about two hours after deposition of the egg and has nothing to do with an embryonic wrapping of cellular origin since the first cleavage occurs only after 2.5 hours (Dittrich and Streibert, 1969). It consisted of layers of protein (chitin) fibrils (Dittrich, 1971). About 44 hours after deposition, perforation organs, first called embryonic stigmata, began to de-

velop (Dittrich and Streibert, 1969) while micropillars extended over a special zone of the intermediate lamella (Dittrich, 1971). Perforation organs, described in detail by Dittrich (1971), were birefringent and nothing but specialized setae (Grandjean, 1948). 48 hours after deposition, the respiratory apparatus composed of the perforation cones associated to the zone covered by micropillars was fully formed and perforation of the egg shell by the cones occurred after 68 hours. Some 44% of the eggs hatched after 76 hours (Dittrich, 1971). After hatching, the intermediate lamella with the perforation cones remained within the egg shell.

If multiple protelattosis occurred in spider mites, two apoderms should coexist within the egg, one belonging to the prelarva and a second belonging to the larva. *Tetranychus urticae* has transparent eggs, 150 μm in size, which allow observation of the complete development under the microscope (Grbić *et al.* 2007a). No multiple calytostases were detected

when breaking eggs near hatching and no element from Dittrich's publications and from recent studies (Grbić *et al.*, 2007; Khila and Grbić, 2007) allows concluding to their existence.

Intervening calyptostase

The observation of pharate individuals at stage S_2 , S_3 and S_4 gave the same results: there was no cuticular structure visible between the external cuticle, the future exuvia, and that of the stase ready to emerge, in light microcopy as well as in TEM (Fig. 3). If there was an intervening calyptostase, a calyptostatic apoderm should be observed between these two cuticular layers.

Comparative chaetotaxy

A first approach consists in comparing the chaetotaxy of leg segments. As noted by Lindquist (1985), the ontogenetic pattern of trochanters affords a ready distinction between immatures and the so-called adults of spider mites since trochanteral IV appears only in the last stage (Table 2). If we compare it to that of Tuckerellidae, two hypotheses are possible. Either the penultimate stage of Tetranychidae, S_3 , is a deutonymph, or it is a tritonymph. Under hypothesis 1, the trochanteral formula of the so-called tetranychid deutonymph is much richer –more plesiomorphic– than in Tuckerellidae. Hypothesis 1 is in contradistinction to Lindquist's (1985) conclusion that the basic pattern of trochanters in Tetranychidae is derived –or apomorphic– compared to that of Tenuipalp-

TABLE 2: Ontogenetic pattern of trochanters in Tuckerellidae and Tetranychidae with distances (d) between homologous stases under hypotheses H_m (multiple protelattosis) and H_p (paedogenesis). Distances are measured using the Manhattan metric.

Tuckerellidae		Tetranychidae		Distances	
Stase	Formula	Stase	Formula	H_m	H_p
(A ₅) Ad	(1-1-2-1)	S ₄	(1-1-1-1)	d(Ad-S ₄) 1	d(TN-S ₄) 1
(A ₄) TN	(1-1-2-1)	S ₃	(1-1-1-0)	d(TN-S ₃) 2	d(DN-S ₃) 2
(A ₃) DN	(0-0-1-0)	S ₂	(0-0-0-0)	d(DN-S ₂) 1	d(PN-S ₂) 1
(A ₂) PN	(0-0-1-0)	S ₁	(0-0-0)	d(PN-S ₁) 1	d(La-S ₁) 0
(A ₁) La	(0-0-0)			$\Sigma=5$	$\Sigma=4$

Stase abbreviation as in Fig. 1.

TABLE 3: Mean distance between homologous stases, d , under different hypotheses. Calculations are detailed for each leg segment and each set of setae.

	H_m	H_p	H_o
Trochanters (*)	5	4	4
Femora	19	15	15
Genua	16	18	18
Tibiae	13	13	13
Tarsi	28	22	22
Epimera	14	8	8
Genitals + aggenitals	7	1	3
Σ	93	74	76
Mean distance (d)	23.25	18.50	19.00

(*) See details in Table 1.

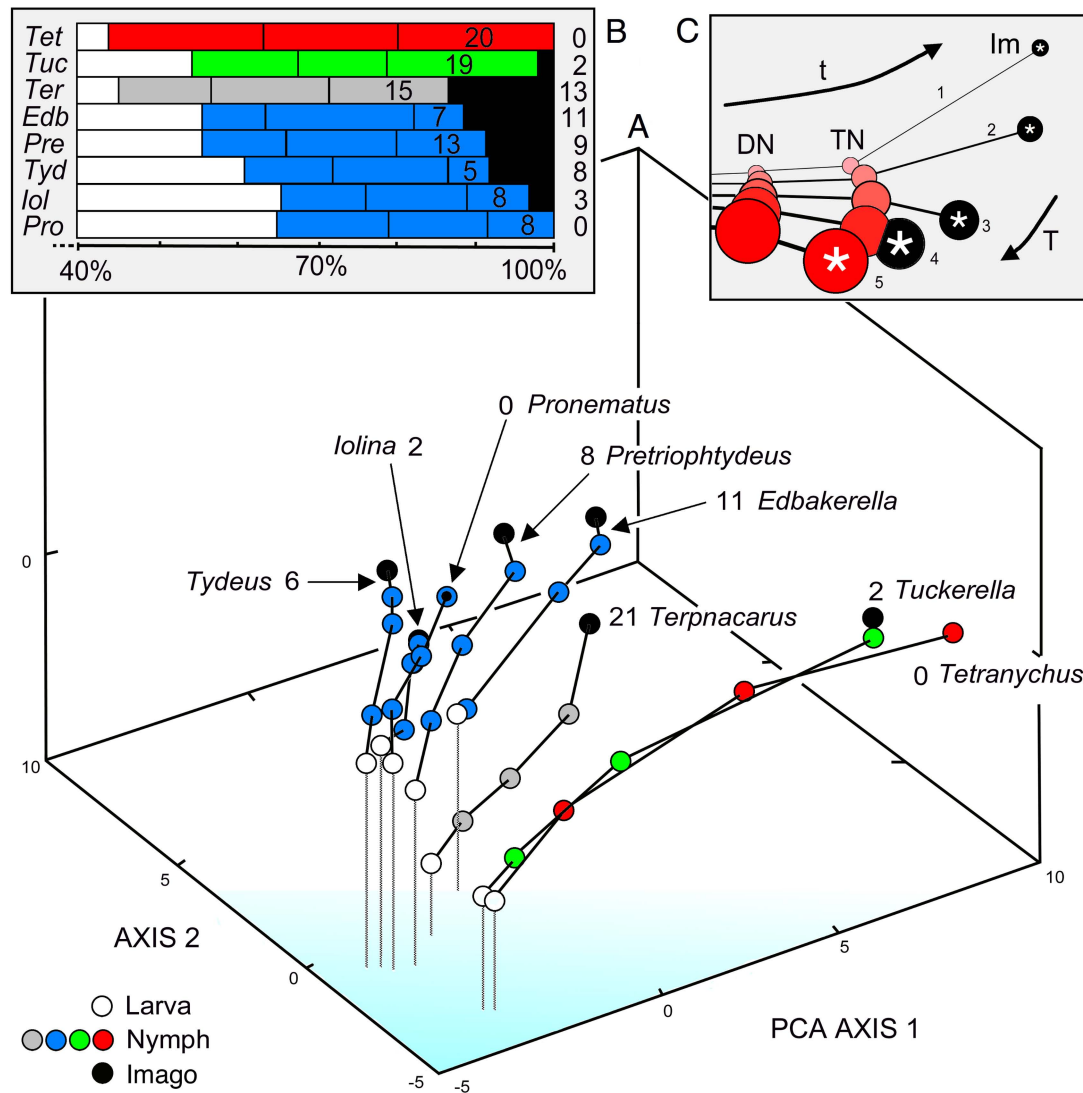


FIGURE 4: Ontogenetic trajectory of *Tetranychus* and *Tuckerella* compared to that of *Terpnacarus bouvieri* (Endeostigmata) and some tydeoid genera. The length of the last segment, i.e. the distance between the imago and the tritonymph, is indicated for each taxon (The length is not necessarily well appreciated on the drawing due to the perspective and use of PCA). In the case of *Pronematus*, both points representing the imago and tritonymph coincide (dotted circle). Insert B illustrates the contribution (in %) of each stage to the trajectories; the imago contribution is given at the right; that of the tritonymph, within the corresponding block. Taxa are identified by the first three letters. Insert C schematizes the progenesis in spider mites, with neoteny (steps 1 to 4) resulting from the shortening of the imago contribution and progenesis (step 4 to 5) when reproduction is taken over by the tritonymph. t: ontogenetic time; T: phylogenetic time; *: adulthood (reproduction). Other abbreviations as in Fig. 1.

idae and Tuckerellidae. Under hypothesis 2, trochanteral chaetotaxy in Tetranychidae is derived –apomorphic– at all levels of ontogeny and ontogenies of both families, Tetranychidae and Tuckerellidae, follow parallel trajectories.

To complete this comparative approach of trochanters, the distance, d , between the chaetotaxy of homologous stases may be estimated under either hypotheses, H_m and H_p (Table 3). If we sum these distances for each family, the total is smaller under hypothesis H_p than under hypothesis H_m . In other words, the ontogenetic trajectory of spider mites, plotted in a 4-dimensional space describing the chaetotaxy of trochanters, is closer to that of Tuckerellidae if we suppose that S_1 is a larva and S_4 a tritonymph.

This approach may be repeated for all leg segments as well as for the epimeral and genital chaetotaxy. Ontogenetic trajectories are then plotted into a 27-dimensional space as explained previously (Fig. 4). The mean distance estimated between homologous stases is again smaller under hypotheses H_p (progenesis) than under hypothesis H_m (multiple protelattosis). This conclusion holds whatever the leg segment studied, but the genua and tibiae (Table 3). The mean distance estimated between homologous stases may be estimated again under hypotheses H_0 (traditional interpretation) and is slightly longer than under H_m (Table 3).

DISCUSSION

The prelarva is a calyptostase

The morphology of the so-called intermediate lamella equipped with perforation cones recalls the prelarval apoderm observed in many mites and in most insects where the perforation cones are usually called egg-bursters.

Dittrich (*in litteris*) replied that the prelarva "is nothing but one phase of the embryonic development" and argued that "the perforation organs do belong to the egg and are specific adaptation to his situation concerning water-stress and the necessity of gas-exchange". Far from negating the existence of a prelarva, such adaptations are indicative

of the occurrence of a calyptostase. Indeed, the respiratory function assumed by the perforation cones in association with micropillars is detailed by Van Impe (1983, 1985). Respiration through special organs is the first function assigned due to a prelarva that is supposed to be "inactive". That all prelarvae equipped with perforation cones respire this way remains to be explored.

This respiratory function has also been reported in insect calyptostases. For instance, the hemipupa of Cecidomyiidae (Diptera), a calyptostase ensheathed in the larva, is equipped with complex spiracles consisting of a truncate cone surmounted by a ring of apertures, each of them surrounded by a pair of radial lip-like ridges. Arising from the cones there are several very stout spines which burst the larval integument covering the hemipupa (Fig. 18 in Wyatt, 1963). Analogies between the two calyptostases, the hemipupa of Cecidomyiidae and the prelarva of Tetranychidae, are straightforward: in both cases, cones perforate the outer envelope while the radial ridges in cecidomyids assume the same role as micropillars in spider mites, allowing air access and circulation. The intermediate lamella of *Tetranychus* is thus, morphologically and functionally, the apoderm of a calyptostatic prelarva. This first step of the post-embryonic development is inactive and disregarded in most studies, even in laboratory conditions (e.g. Baker and Wharton, 1952; Grbić *et al.*, 2007; Khila and Grbić, 2007). Protelattosis, *i.e.* the occurrence of an inhibited state at the beginning of ontogeny, is widespread in mites. Nearly all prelarvae are calyptostatic; in a few cases, they are elattostatic.

Hypothesis H_m : multiple protelattosis

Multiple protelattosis, *i.e.* the occurrence of several elatto- or calyptostases at the beginning of ontogeny, is common in spiders, with two to four inhibited stases (André and Jocqué, 1986), and in pseudoscorpions, with two to three inhibited stases (Grandjean, 1938a; André and Jocqué, 1986). Generally speaking, the number of successive calyptostases in Arachnida tends to increase from primitive to more evolved taxa. The same trend is observed in insects. A priori, multiple protelattosis

was thus an attractive hypothesis to explain the peculiarity of ontogeny in spider mites. However, neither the study of the eggs, nor the comparative analysis of chaetotaxy does support this hypothesis. Multiple protelattosis, hypothesis H_m , is thus rejected.

Hypothesis H_i : intervening calyptostase or metelattosis

Metelattosis is also common in mites. So far, it has been possible to detect intervening calyptostases even in ontogenies that comprise multiple calyptostases. This is the case of speleognathin Ereyneidae: the three nymphs are all calyptostasic and remain ensheathed in the imago (Fain, 1972 – Fig. 1I). Several intervening calyptostases are also known to occur in the ontogeny of Hypoderidae (the larva, proto-, and tritonymph) (Fain and Bafort, 1967; Fain, 1967) and Erythraeidae (the proto- and the tritonymph) (Grandjean, 1957, 1959). These calyptostases were recognized either through the presence of an apoderm or pharynx detected in light microscopy. In Tetranychidae, no apoderm indicating the existence of an intervening calyptostase was detected. Van Impe (1985) and André (1988) hypothesized that the protonymph might be calyptostasic since the first nymph of Tetranychidae does not present the denudation of leg IV typical of protonymphs. Their hypothesis does not hold as there is no intervening calyptostase –no metelattosis *sensu* Hammen (1975, 1980)– in the course of ontogeny in Tetranychidae. Hypothesis H_i is thus rejected.

Hypothesis H_o vs H_p

The comparative study of chaetotaxy in Tuckerellidae and Tetranychidae suggests that the first active stage of spider mites is a larva followed by a protonymph. However, we do not yet know the real identity of the last active stage. Provided that hypotheses H_m and H_i have been rejected, both hypotheses H_o and H_p still hold. In the latter case, the oldest stage in the ontogeny of spider mites is a paedogenetic tritonymph (Fig. 1H); in the former, it is an imago (Fig. 1B). In Tuckerellidae, the leg chaetotaxy of the tritonymph is identical to that

of the imago. The only difference in the chaetotaxy of the last two stases concerns the genital area: La (0) PN(0-1) DN(0,2) TN(0,4) Ad (0,2,4). A comparison with Tetranychidae, with genital formulae La (0) PN(0,1) DN (0,2) S4(0,3), reveals that the genital chaetotaxy of the last tetranychid stage is closer to that of the tritonymph than that of the imago. This argument is counterbalanced by the presence of male solenidia in the last stage of Tetranychidae, which recalls those found in male Tuckerellidae. The argument is also weakened by the presence of similar formulae in the imago of other families (e.g. in Pronematinidae, lolinidae...).

The quantitative method used to test H_o vs H_p meets the same problem since the chaetotaxy of the two last stases in Tuckerellidae differs only in the genital formulae. The mean distances between two homologous stases vary little depending on the hypothesis, H_o vs H_p (Table 3). There is a small advantage in favor of H_p , which results from the differences in genital chaetotaxy explained in the previous paragraph.

A last approach consists in comparing the Tetranychoidae to another super-family the ontogenetic patterns of which are well known (Fig. 4). The similarity between the tuckerellid tritonymph and imago (in other words, the short length of the last segment of the ontogenetic trajectory) resembles that found in some Tydeoidea: the more evolved is the genus, the least is the contribution of the imago to the trajectory. In other words, the length of the last segment of the ontogenetic trajectories tends to get smaller and smaller until the imago coincides to the tritonymph as in *Pronematus* (step 4 in Fig. 4 Insert C). The same trend is observed in the ontogenetic trajectory of Tetranychoidae. In Tuckerella, the imago contribution to the trajectory is only 2% and becomes null in *Tetranychus* (Fig. 4B). The situation in *Tetranychus* is similar to that of *Pronematus*, except that the imago is missing. Indeed, if we suppose that the last stage of *Tetranychus* is a tritonymph, its contribution is similar to that of the tritonymph in *Tuckerella* (20% vs. 19%) in the same way the contribution of the tritonymph of *Pronematus* is similar to that of *lolina* (8% in both taxa) (Fig. 4, Insert B).

Supposing that the last stage of *Tetranychus* is

an imago meets two other objections. The first results from the comparative study of ontogenetic trajectories. Indeed, the last stage of *Tetranychus* contributes to 20% of the length of the trajectory; such a high contribution has never been observed in any imaginal stage. A second objection is raised by the implicit absence of the tritonymph. Such an absence in *Tetranychus* seems little realistic when compared to endoparasitic species. Indeed, even endoparasitic species with a sophisticated ontogeny as that observed in Speleognathinae, have kept all the nymphs: all three are reduced to calyptostases. This was interpreted as an indicator of developmental constraints by André (1992). Hypothesis H_0 is thus rejected in favor of hypothesis H_p .

Paedogenesis in *Tetranychus* and actinotrichid mites

Identifying the last stage in *Tetranychus* as a tritonymph implies that it is paedogenetic. Is this acceptable? Paedogenesis in actinotrichid mites has been reported in various groups, in Podapolipidae by Volkonsky (1940), Pyemotidae by Rack (1972), Tenuipalpidae by Baker (1979) and Cheyletidae by Atyeo *et al.* (1984). The penultimate case is of special interest as Tenuipalpidae are part of Tetranychoida. The missing link is however provided by Ochoa (1989) who reported paedogenetic tritonymphs coexisting with imaginal females in *Tuckerella knorri*. This particular ontogeny is summarized in Fig. 1G and links ontogenies A (usually observed in Tuckerellidae, Beard and Walter, 2005) and H (that of *T. urticae*). A second intermediate case is provided by *Tuckerella saetula* and *T. cf. pavoniformis* in which the male directly emerges from the deutonymph (Beard and Ochoa, 2010).

In the context of the stase theory, Hammen (1975) called allassostasy the variations in the number of stases and the process of losing a stase during the ontogeny was named meristasy by André (1988). The ontogeny in Tetranychidae is thus characterized by the meristasy of the imaginal stage.

Adult state, adult instar and adult stase

The current terminology of post-embryonic development is confusing and bewildering (Breene, 2003)

as it pertains to three approaches. The first approach is related to form, state and, sometimes, function. A mite larva is defined or described by the presence of six legs. Adults are defined by genitalia and their function is reproduction.

A second and traditional framework for the description of arthropod development takes the molt-to-molt interval as the fundamental unit of periodization (Minelli *et al.*, 2006). In some cases, this number is variable and no homology between instars is possible, the example of Argasidae is illustrative. The instar terminology refers to the same words as the form approach: larva and adult.

A last and third approach emphasized by Grandjean (1938a, 1970) and André (1988, 1989) is related to stases. Stases are based on idionymy, it is thus possible to homologize them. Again, the same words are used to define ontogenetic levels, larva and adults.

As a result, some confusion revolves around the use of the term "adult". If by tradition the 6th level of ontogeny is named the adult -or more precisely the adult stage, we have to admit that the adult state -or adulthood- is likely to occur at an earlier level. The confusion is not new and some entomologists prefer to use the term "imago" to refer to the last step of the usual ontogeny; unfortunately, this usage is not widely accepted. We thus propose to rename the 6th level of mite ontogeny and to call it the imago (Im). This designation of the final step in ontogeny is in agreement with dictionaries (Séguy, 1967; Oxford Dictionaries, 2010). The text of this publication has been revised accordingly as well as figures.

Heterochrony and development in arachnids

Three evolutionary changes involved in the ontogeny of mites are illustrated in Fig. 1H-I-J. The first change concerns stases that are calyptostasic, even in parasitic species. Apparently, there are just two active instars, the larva and the imago in ereynetid Speleognathinae (Fig. 1I). There is however neither a shortening of ontogeny when stases are considered, nor an "omission" as misunderstood by Matsuda (1979). A second case concerns a real missing stage: there is no deutonymph in some

Astigmata, even when permanent rearing is available. The mite that follows the protonymph in ontogeny is homologized with a real tritonymph (Fig. 1J). Last, *T. urticae* shows a third change, there is a real truncation of the post-embryonic development (Fig. 1H). The number of active instars is the same as in the second case but the reduction occurs at another level of development.

As explained in a former section, paedogenesis in actinotrichid mites is common. The paedogenesis described in other actinotrichid mites is however different from what happens in Tetranychidae. In other mites, eggs or larvae were observed in the idiosoma of immature stases. As the latter lack a genital orifice, the former are supposed to escape by rupture of the integument of the maternal idiosoma (Evans, 1992) as is the case for some insects as the genus *Miastor* (Diptera, Cecidomyiidae). In insects, these paedogenetic females reproduce most often by parthenogenesis, ovaries proliferate and give rise to haemocoelous embryos that develop into young larvae feeding on the maternal tissues. This method of reproduction is fundamentally different from the usual process of laying eggs through the oviducts observed in Tetranychidae tritonymph but this process might precede and indicate a truncation of development. The use of this term prompts another comment. Paedogenesis is part of the heterochrony conceived as any change in rate or timing of ontogeny. Gould (1977) gives a historical overview of the origin and development of this terminology. In Tetranychidae, there is a nymphal reproduction via precocious reproductive development. This peculiarity does not obey the original definition of paedogenesis, a term coined by Baer (1866; cited in Gould, 1977) who published only a few minor, mostly trivial papers on insects (Groeben, 1993). Baer restricted his definition to cases of parthenogenesis in insect larvae structurally unable to copulate. Hamann (1891; cited in Gould, 1977) extended Baer's term to include all precocious development. Tetranychid development conforms to Hamann's meaning and also obeys the definition of progenesis, a term coined by Giard (1887; cited in Gould, 1977) to designate accelerated reproductive development. This term is even used

by Gould (1977) to describe the development of the mite *Siteroptes graminum*. Progenesis in trematodes, another taxon with molts, encompasses all cases where a larval stage produces viable eggs (Lefebvre and Poulin, 2005).

Another distinction is often made by entomologists. They speak of prothetely when the adult develops too "early" (the quotes are by Gould, 1977), perhaps at a precocious molt. In this context, prothetely is opposed to metathetely. "When an insect becomes a neotenus adult after undergoing a normal or more than normal number of molts, the condition is metathetely; whereas it becomes a neotenus adult after undergoing less than the normal number of molts, the condition is prothetely" (Matsuda, 1979: 139, definition recalled in Pearson, 2004: 73). This approach is based on the number of molts and not on development levels.

Pearson (2004) laments the confusion surrounding heterochrony. This is still true. If this terminology applies to mite development, it is little relevant to the stase approach. There is no reference to Grandjean's publications in the references listed by Gould (1977).

CONCLUSIONS

All elements (study of the chaetotaxy, comparison with related families (Tuckerellidae), comparison with other mites such as the Tydeoidea) lead to the same conclusion. The development of *Tetranychus urticae* comprises five stases (among which four are active) and the tritonymph has the reproduction in charge. The post-embryonic development may be summarized this way: prelarva (calyptostase), larva (six-legged stase), protonymph (eight-legged stase), deutonymph (eight-legged stase), tritonymph (with paedogenesis, *i.e.* precocious development of sexual maturity). Tetranychidae are evolved mites with typical colonizing and 'opportunistic' features (Lebrun *et al.*, 1991). The occurrence of the imaginal meristasy resulting from paedomorphosis is not surprising in such a taxon. Within the Prostigmata, there is marked trends towards a reduction in chaetotaxy, especially in imagines and secondarily in tritonymphs (as opposed to taxa characterized by

neotrichy), and towards a shortening of the ontogenetic trajectories. The neotenus features (in terms of arrest of development of the chaetotaxy) have become increasingly pronounced in Prostigmata and seem to indicate a phylogenetic neoteny. This trend contrasts with that observed in Oribatida in which the imago has become heteromorphic in higher taxa (Euoribatida).

Tetranychidae offer an illustrative example of the passage from an ontogeny with five active stases to another with only four, or, if we consider also the calyptostases, from an ontogeny with six stases to another with five. The meristasy of 5 and 6th levels probably applies also to Eriophyidae with two active stases following a calyptostatic prelarva described by Shevchenko (1961).

It may be of interest to recall that the fundamental number of stases is six in actinotrichid mites, *i.e.* the same number as in Pseudoscorpions and Ricinulei. This has even been an argument to combine the Acari and the Ricinulei under the name Acaromorpha (see the review by Weygoldt, 1998). Among Anactinotrichida, Opiliocariformes and maybe Holothyrida have also six stases. Mesostigmata have an ontogeny with only four active stases. That they have followed the same process as Tetranychidae in reducing the number of stases remains to be explored. Meristasy of the terminal stage is also known in Pseudoscorpions in which the tritonymphal stage is adult (Judson, pers. comm.).

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