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Convergent evolution of defense mechanisms in oribatid mites (Acari, Oribatida) shows no "ghosts of predation past"

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ABSTRACT

Oribatid mites are diverse and abundant terrestrial soil arthropods that are involved in decomposition of organic matter and nutrient cycling. As indicated by fossils starting from the Devonian, they evolved varied mechanisms and structures for defense from predators. We investigated four of these defensive structures (ptychoid body, hologastry, mineralization and opisthonotal glands) and used ancestral character state reconstruction to determine whether they evolved convergently and how many times this may have happened. Phylogenetic trees based on 18S rDNA were constructed for 42 oribatid mite species and two outgroup taxa using likelihood and Bayesian algorithms. The results suggest that at least three of the four defensive structures evolved convergently several times; for opisthonotal glands convergent evolution remains equivocal. This high level of convergence indicates that predation has been an important factor throughout the evolution of oribatid mites, contributing to morphological diversity and potentially also to species richness, as there are indications that some taxa radiated after the evolution of defense structures. Despite the ancientness of oribatid mites, defense structures seems to have been rarely lost, suggesting that they still are functional and necessary to reduce predation, rather than being 'ghosts of predation past'.

1. Introduction

The importance of particular selection pressures can be inferred from phylogenies that indicate convergent evolution. Similar ecological pressures can result in similar adaptive solutions (Johannesson, 2003) and this appears to have been common (Conway Morris, 2005). Adaptive convergence often has been viewed as proof of evolution itself and therefore has received special attention in reviews and textbooks (Fain and Houde, 2004; Jones and Holderied, 2007; Gillespie et al., 2003; Miller et al., 2010). In oribatid mites (Acari) bark living (Maraun et al., 2009), aquatic life (Schatz and Behan-Pelletier, 2008) and a predatory mode of nutrition (Maraun et al., 2011) all appear to have evolved convergently.

Oribatid mites (Acari; Oribatida) are small (mostly 150–1000 μm), abundant arthropods that predominantly live in soil, but some species occupy more insular microhabitats, such as decomposing woody substrates, mosses, lichens and the bark of trees. Most species feed on dead organic material and fungi, but specialists or opportunists may also utilize algae, lichens, bacteria, nematodes and remains of small invertebrates (Schneider et al., 2004; Norton and Behan-Pelletier, 2009; Maraun et al., 2011). They have unusual life histories for such small animals: they grow slowly, lay relatively few eggs and are long lived. With such features oribatid mites were considered K-strategists until Norton (1994, 2007) proposed that these traits, rather than reflecting a 'strategy', are consequences of low-quality diets as compared to their predaceous arachnid ancestors. In this conceptual model, the slow accrual of resources for growth and reproduction requires an extended development and long adult life, putting a selective premium on adaptations for defense from predators.

The oldest certain fossils of oribatid mites are from the Devonian (Norton et al., 1988), and molecular clock estimates even date their origin in the Precambrian era (ca. 571 ± 37 mya; Schaefer et al., 2010). Early oribatid mite species were at most weakly sclerotized, but even these possessed large dorsal setae, which in extant primitive species are erected to create defensive space (Norton et al., 1988; Subías and Arillo, 2002). During a relatively short period of time oribatid mites evolved a more sclerotized cuticle and a wide array of more specialized defense mechanisms (Norton, 2007). These can be grouped as cuticular mineralization (for mechanical hardening), defensive hairs (either as protective plates or as 'spacers' to keep predators at a distance), waxy cuticular secretions or adherent debris (visual or tactile camouflage, surface roughening or spacers), protective tecta (hard, roof-like projections

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over vulnerable articulations), defensive body forms (structural organizations that allow protection of vulnerable regions) and defensive glands (Raspotnig, 2010). Each of these structures appears to be ancient, likely having evolved during the Paleozoic.

Often, the defensive traits themselves have been foci for classifications or phylogenetic hypotheses about oribatid mites. For example, the taxon Ptyctima was proposed by Oudemans (1906) for all species exhibiting the defensive body form called ptychoidy (see below). Grandjean (1969) considered ptychoidy to exhibit convergence, but some taxonomists only partially recognized his ideas (e.g. Subías, 2004). Haumann (1991) recognized the taxon Holonota to include all taxa with a hardened, unsegmented dorsal shield (i.e., that exhibit 'hologastry'); some authors still follow this organization, but Norton (2010) viewed it as a convergent trait. Frequent convergence can result in a mosaic-like distribution of characters (Smith et al., 1995; Woas, 1998; Nevo, 1999) that confounds attempts at phylogenetic classifications based on phenotypes.

We used molecular methods—independent of morphology—to test existing hypotheses of evolutionary convergence in defensive traits, including an analysis of ancestral character states, and to detect other possible examples. Molecular phylogeny-based methods have been shown to be a powerful tool for inferring the evolutionary history of morphological traits in oribatid mites (Schäffer et al., 2010). We focus on four traits: (i) ptychoidy, i.e., the general body form involving the ability to retract legs and their support structures into a sclerotized or mineralized 'abdomen', producing a seed-like appearance with the hardened anterior plate (prodorsum) forming a protective cap (Sanders and Norton, 2004), (ii) mineralization, i.e., the incorporation of minerals, such as calcium carbonate, calcium phosphate and calcium oxalate (Norton and Behan-Pelletier, 1991a), for hardening of the cuticle, but potentially also reducing the risk of desiccation (Norton and Alberti, 1997), (iii) hologastry, i.e., the fusion of dorsal sclerites ancestrally being separated by articulations thereby forming a single unified notogaster, and (iv) opisthonotal glands, i.e., 'abdominal' glands secreting repellants against predators, alarm pheromones and antimicrobial substances (Raspotnig et al., 2003; Sakata and Norton, 2003; Raspotnig, 2010).

Adults of extant oribatid mites in soil have been proposed to suffer little from predation and consequently have been postulated to live today in 'enemy-free space' (Peschel et al., 2006). Since the group is so ancient, it is reasonable to ask if their defensive structures still are necessary or if they represent 'ghosts of predation past'. Such 'ghosts' are defensive characters that have been retained despite the fact that potential predators of their bearer no longer exist, or have switched to other prey (Peckarsky and Penton, 1988; van Moorter et al., 2009).

2. Materials and methods

2.1. Taxon sampling

The taxon set used in this study represents 44 species (Table 1), including 42 oribatid mite species. As these are members of the Acariformes (=Actinotrichida), we used two species of the Parasitiformes (=Anactinotrichida) as outgroups: *Amblyomma sphenodonti* (Ixodidae) and *Opilioacarus texanus* (Opilioacaridae). Our goal in selecting species was twofold: (1) to include multiple species having each of the four traits, being certain to represent groups that are relevant to hypotheses of convergence present in the literature, and (2) to represent enough general oribatid mite diversity to provide a robust overall phylogeny. A priori, species were assigned to one of the six major groups of oribatid mites following the classification of Grandjean (1969) as modified by Norton and Behan-Pelletier (2009).

The ptychoid body form occurs in both Mixonomata and Enarthronota; four and five species, respectively, having this form were sampled, along with other members of these groups that are not ptychoid. Hologastry characterizes all members of Mixonomata, Desmonomata and Brachypylina, each of which is abundantly sampled, and some of Enarthronota (three of the many species sampled). Opisthonotal glands are found in all known Desmonomata, Brachypylina, and the species-poor Parhyposomata, as well as most Mixonomata, but are absent from Palaeosomata and Enarthronota; we sampled three mixonomatans that lack the gland and six that have it.

GenBank provided sequences for 34 of the sampled species (Table 1). Sequences from 10 species were newly obtained during this study. Specimens were collected from litter and soil samples using standard heat extraction (Kempson et al., 1963) and stored in 75% EtOH at −20 °C.

2.2. DNA extraction, PCR and sequencing

Template DNA was extracted from 1 to 20 individuals using the DNeasy Blood and Tissue Kit (Qiagen, Hilden, Germany) following the manufacturer's protocol with final elution in 30 µl H_2O and stored at −20 °C. Amplification of the 18S region was performed in 25 µl volumes containing 12.5 µl HotStarTaq Mastermix (2.5 units of HotStarTaq polymerase, 200 µM of each dNTP, 15 mM $MgCl_2$; Qiagen, Hilden, Germany), 5 µl of template DNA, 1 µl of each primer (100 pM) and 5.5 µl H_2O. Primers for PCR were 5′-TACCTGGTTGATCCTGCCAG-3′ (forward) and 5′-TAATGATCCTTCCGCAGGTTCAC-3′ (reverse) (Domes et al., 2007). The PCR protocol consisted of an initial activation step at 95 °C for 15 min, 35 amplification cycles (95 °C for 45 s, 57 °C for 60 s, 72 °C for 60 s) and a final elongation step at 72 °C for 10 min. All PCR products were visualized on a 1% agarose gel, purified with the QIAquick PCR Purification Kit (Qiagen), and sequenced by Macrogen Inc. (Seoul, South Korea), using the additional sequencing primers 18S554f 5′-AAGTCTGGTGCCAGCAGCCGC-3′, 18S1282r 5′-TCACTCCACCAACTA AGAACGGC-3′, 18S1150f 5′- ATTGACGGAAGGGCACCACCAG-3′ and 18S614r 5′- TCCAACTACGAGCTTTTTAACC-3′ (Domes et al., 2007).

2.3. Alignment and phylogenetic analysis

Phylogenetic analyses were carried out using the multi-copy gene 18S rRNA. Sequences were assembled and ambiguous positions were corrected in Sequencher 4.8 (Gene Codes Corporation, Ann Arbor, Michigan, USA). A preliminary alignment was generated using ClustalX v1.8 (Thompson et al., 1994) with the multiple alignment parameters gap opening = 20 and gap extension = 0.1 and cut to a uniform length of 2127 bp. From the alignment a NJ tree was generated in SeaView v4.2.3 (Gouy et al., 2010) and used as a guide tree to correct for long branch artifacts in the initial guide tree to generate a new alignment with the same parameters in ClustalX. The final alignment had a length of 1991 bp; it has been published in TreeBASE under the study accession number S12353 (www.treebase.org). The evolutionary model parameters were determined with Modeltest 3.7 (Posada and Crandall, 1998), using a hierarchical likelihood ratio test (hLRT). The best fit model for sequence evolution for 18S was TrN + I + G. Phylogenetic trees were constructed using Bayesian inference in MrBayes 3.1.2 (Huelsenbeck and Ronquist, 2001) and Maximum likelihood (ML) in RAxML 7.0.4 (Stamatakis, 2006). Bayesian trees were calculated using JC (nst = 1) and GTR + I + G (nst = 6; rates = invgamma) models with three independent runs of three million generations and four chains per run; rate matrix and base frequencies were estimated. We used JC and GTR + I + G in MrBayes since GTR + I + G