

Arthropod trace diversity on fossil leaves from the mid-Cretaceous of Negev, Israel

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ABSTRACT

This study of parasitoid marks on leaf impressions (phyllostigmas) is based on fossil plant material from the Albian to Turonian of central and southern Negev. On account of widespread homeomorphy of phyllostigmatic structures produced by various organisms, their taxonomic assignments are but tentative emphasizing similarities of habits, evolution of which is recorded in the first place. The mid-Cretaceous phyllostigmas represent specialized habits of oviposition, external feeding, gall making and mining, their diversity being greatly enhanced by the advent of angiosperms. The Albian phyllostigmas mostly occur on gymnosperm leaves, the contemporaneous angiosperm leaves being infested with egg sets of Odonata alone, indicating a waterside habitat. Angiosperm leaves became more frequently stigmatized during the Cenomanian followed by the major diversification burst of parasitic arthropods in the mid-Turonian. The widespread occurrence of procecidia, the prevalence of organoid galls, some drastically damaging to the leaves, and of extensive blotch mining are evidence of a relatively low co-adaptation level in the mid-Cretaceous plant – arthropod communities. “Weakness parasitism” is suggested by the frequent on leaf co-occurrence of various phyllostigmas. At the same time, the first appearance of mine and gall predators might have invoked an incipient top-down regulation of parasitoid systems. In this respect, the coastal and inland plant – arthropod communities show different levels of evolutionary advancement.

KEY WORDS: Plant – insect co-evolution. Fossil galls. Fossil mines. Paleoecology. Cretaceous. Israel.

INTRODUCTION

Traces of arthropod feeding and habitation on fossil leaves (phyllostigmas) add to the diversity of contemporaneous fossil faunas, in particular because trace makers (both miners and gall inducers) are rarely if at all represented in the body fossil assemblages. Yet classification of traces primarily reflects the diversity of habits, being indirectly, and sometimes ambiguously, related to taxonomic diversity. In the trace studies, parasitic habits are inferred from the structures these habits induced or produced. It must be said from the very beginning that our comparisons with phyllostigmas of modern arthropods do not imply taxonomic affinities, but indicate similarity of behavioral traits that has evolutionary history of their own.

Structural diversity of trace assemblages conveys the diversity of parasitic behavior, evolution of which we are tracing in the first place. Parasitic behavior is regulated by the host responses on the one hand, and by the secondary parasites (parasitoids) and predators on the other, therefore providing evidence of ecosystem structure and functioning.

Our data refer to a major restructuring stage in the history of terrestrial ecosystems brought about by the advent and rise of angiosperms. In the middle of Cretaceous period already, these newcomers have surpassed all

the other groups of higher plants in the diversity of their foliar and floral structures, as well as in plasticity of their growth forms. These developments were matched, and perhaps enhanced, by the burst of adaptive radiation in the leaf parasites providing ample evidence of plant – arthropod co-evolution.

MATERIAL AND METHODS

In the Negev Desert, the Aptian to Middle Albian deposits are non-marine or marginally marine, with occasional normal marine intercalations overlain by the platform carbonates with periodic influxes of terrestrial material. Stigmatized leaves are recorded over the sequence of Aptian to mid-Turonian plant bearing horizons (Fig. 1).

In the Makhtesh Ramon depression of central Negev, the Upper Hatira Formation comprises variegated sandstones, clays and marly shales with the Albian ammonites *Knemiceras* Böhm. in the lower horizon. It is overlain by the Upper Albian – Cenomanian carbonates. Fossil plants are represented by ferns, ginkgophytes, conifers and angiosperms forming several plant communities (Silantieva & Krassilov 2006). Few narrow-leaved angiosperms (or possibly pro-angiosperms of *Acaciaephyllum* type) bear sets of inserted eggs.

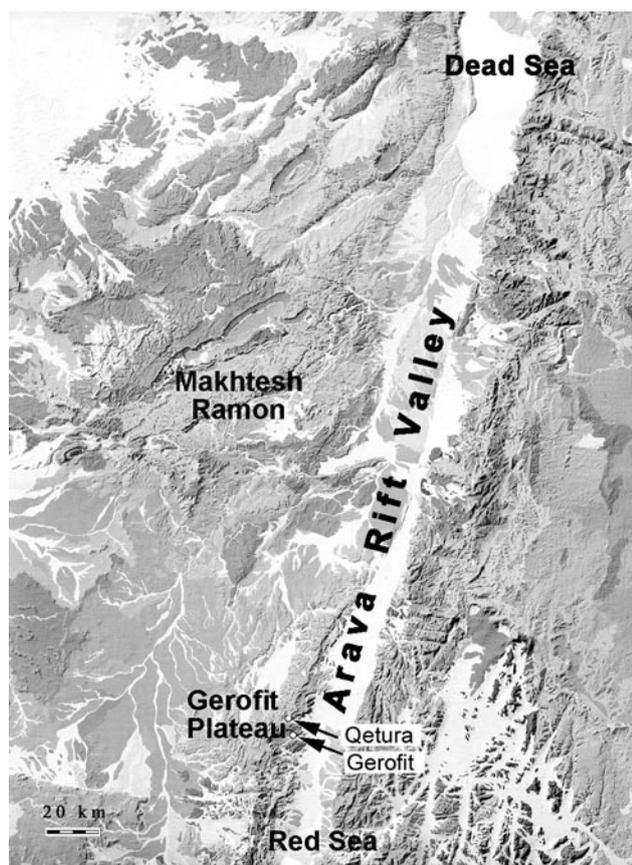


Figure 1. Map showing the Cretaceous plant and insect localities of the Negev.

No arthropod traces were found in the Cenomanian of Israel, but a few were reported from the Nammoura locality in the Mont-Liban district of Lebanon (Krassilov & Bacchia 2000). The flora is essentially angiospermous, with few ferns and gymnosperms. A leaf of gymnospermous *Pseudotorrelia* is mined and occasional angiosperm leaves show insect cut-outs.

The mid-Turonian plants and insects of Arava Valley, southern Negev come from two localities at the mouth of Gerofit Wadi about 60 km north of Eilat and in the Qetura Wadi 2.2 km further north, 4 km before the mouth. Both represent one and the same plant-bearing horizon in the Upper Shale Member of the Ora Formation. Their age assignment is based on the mid-Turonian ammonites found both in the underlying Vroman Bank limestones and the overlying carbonates of Gerofit Formation (reviewed in Krassilov *et al.* 2005). The plant-bearing sequence starts with cross-bedded sandstones followed by gray shales and then by red (crimson) shales. It is overlain by an alternation of dolomites and variegated shales with oyster beds near the base and with gypsum and anhydrite above. The flora is presently the most representative not only in Israel but for the northern Gondwana realm as a whole. It comprises about 50 species of angiosperms belonging to the orders of modern dicotyledons Ranunculales, Nymphaeales,

Nelumbonales, Trochodendrales, Hamamelidales, Juglandales, Rosales, Myrtales, and Sapindales, monocotyledons Najadales, Pontederiales, Arales, Cycolanthales, Arecales, and Typhales, and an extinct *Gerofitia* group, probably proangiospermous.

The locality is remarkable in abundance of oviposition, surface feeding, mining, and gall construction traces on leaves, constituting perhaps the most diverse regional trace assemblage (Krassilov 2007). Insect body fossils are relatively rare, representing 16 species of nine extant and one extinct family (Dobruskina *et al.* 1997 and under study). In the collection of about 1,500 specimens of terrestrial and aquatic angiosperms, most of the leaves are parasitized. The leaves are preserved as reddish ferruginous mineral films deposited on the leaf surface and beneath the cuticle. The galls and mine remains are conspicuous due to their usually brighter coloration and the darker hues of the surrounding callus. The parasitic structures were studied and photographed under stereomicroscope LEICA MZ6 and digital camera LEICA DFC320. Mineral films with egg remains were transferred to varnish and studied in transmitted light.

The collection is deposited in the Institute of Evolution, University of Haifa, Israel, depository numbers IMR and IG1.

RESULTS

About 60 morphological types of phyllostigmas are recognized in the Cretaceous trace assemblages, including egg scars, procecidia, organoid and prosoplasmatic galls, and mines of various configuration types (Figs 2, 3). In the Albian assemblages, the most frequently attacked host plants are gymnosperms and *Acaciaephyllum*-like narrow-leaved (pro)angiosperms. In the Turonian assemblages, for which a number of coastal to inland plant communities are recognized (Krassilov *et al.* 2005), they are the compound *Dewalquea* and *Eudebeya* leaf morphotypes of the coastal, supposedly mangrove, community, the floating and emergent *Nelumbites*-type leaves of aquatic plant, and the riparian to dryland broadleaves *Eucercidiphyllites*, *Plataneriva* and *Platydebeya*.

Eggs

The egg scars are insertions with more or less prominently raised, apparently callous, margins implying wound reaction. Solitary insertions were much more frequent at this evolutionary stage than nest insertions, which were found in two occasions only. A single example of structurally preserved egg shells shows a simple, with incipient differentiation, intrachorionic transpiration meshwork (Figs 3K, L), which is commonly considered as more primitive than the complex intrachorionic and plastronic systems (Hinton 1981).

The Albian egg set shows a zigzag pattern (Fig. 2A) characteristic of Odonata and assigned to “Coenagrionid Type” by Helmund & Helmund (1996 and elsewhere)

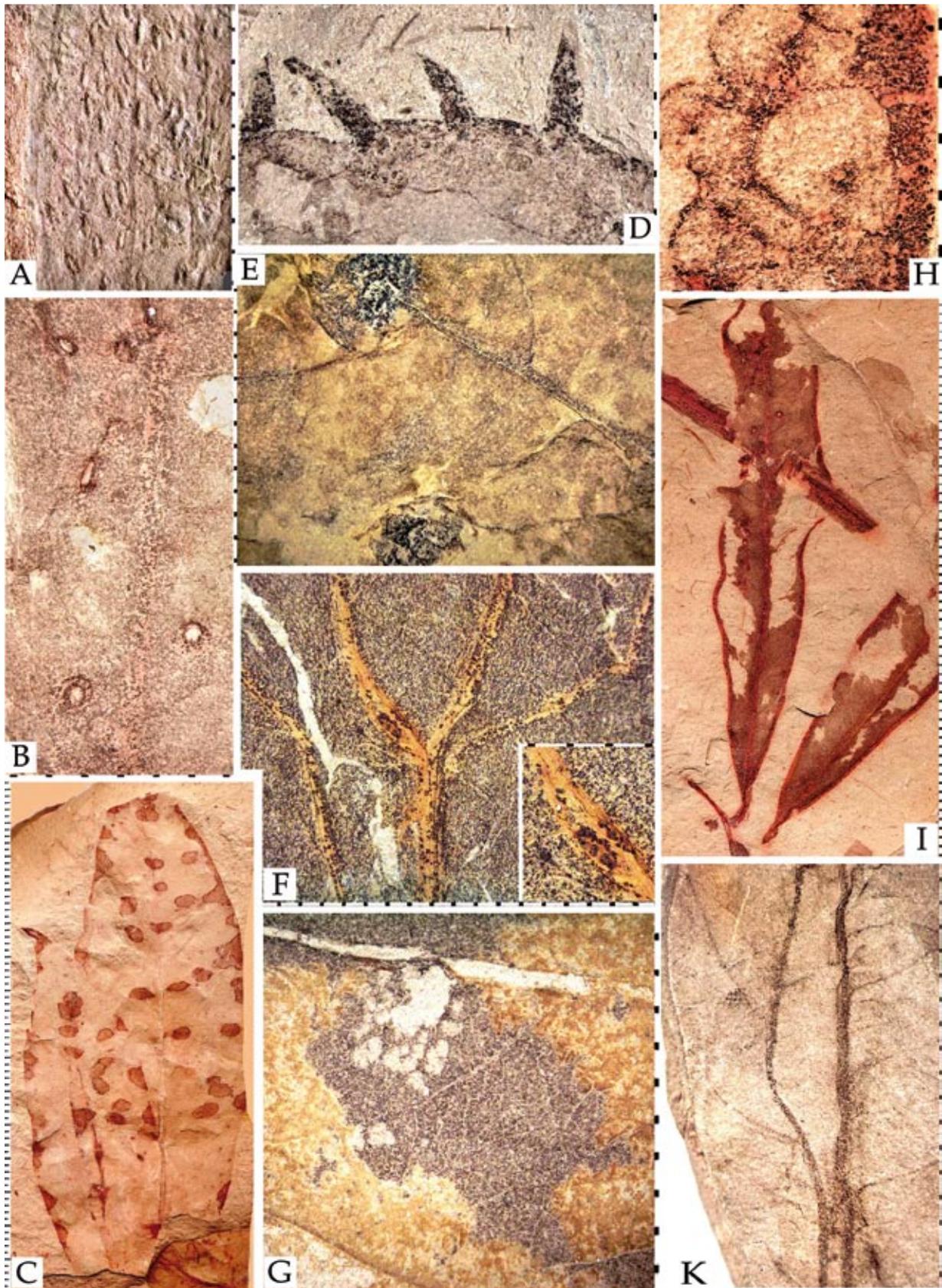


Figure 2. Insect traces on leaves from the Albian of Makhtesh Ramon (A) and Turonian of Geroftit (B – K); (ruler point 1 mm). A. IMR-A2-303, egg set of Odonata on *Acaciaephyllum*-type leaf. B. IG1-887, postulate procecidia on *Dewalquea geroftica*. C. IG1-893, lenticular psyllid galls on *Eudebeya angusta*. D. IG1-227, *Ceratoneon* galls on *Dewalquea geroftica*. E. IG1-403, *Cephaloneon* gall on *Menispermites cuspidentatus*. F. IG1-444, midrib branching caused by a pit gall (on insertion) on *Platanervia integrifolia*. G. IG1-31, trichomate fitzgall area of eriophyid mite on *Retrodewalquea pendens*. H. IG1-479, agglomerated aphid gall of larger central and smaller radial bulges on *Dewalquea geroftica*. I. IG1-887, margin roll galls in association with other phyllostigmas on *Dewalquea geroftica*. K. IG1-232, Midrib splitting gall on *Dewalquea geroftica*.

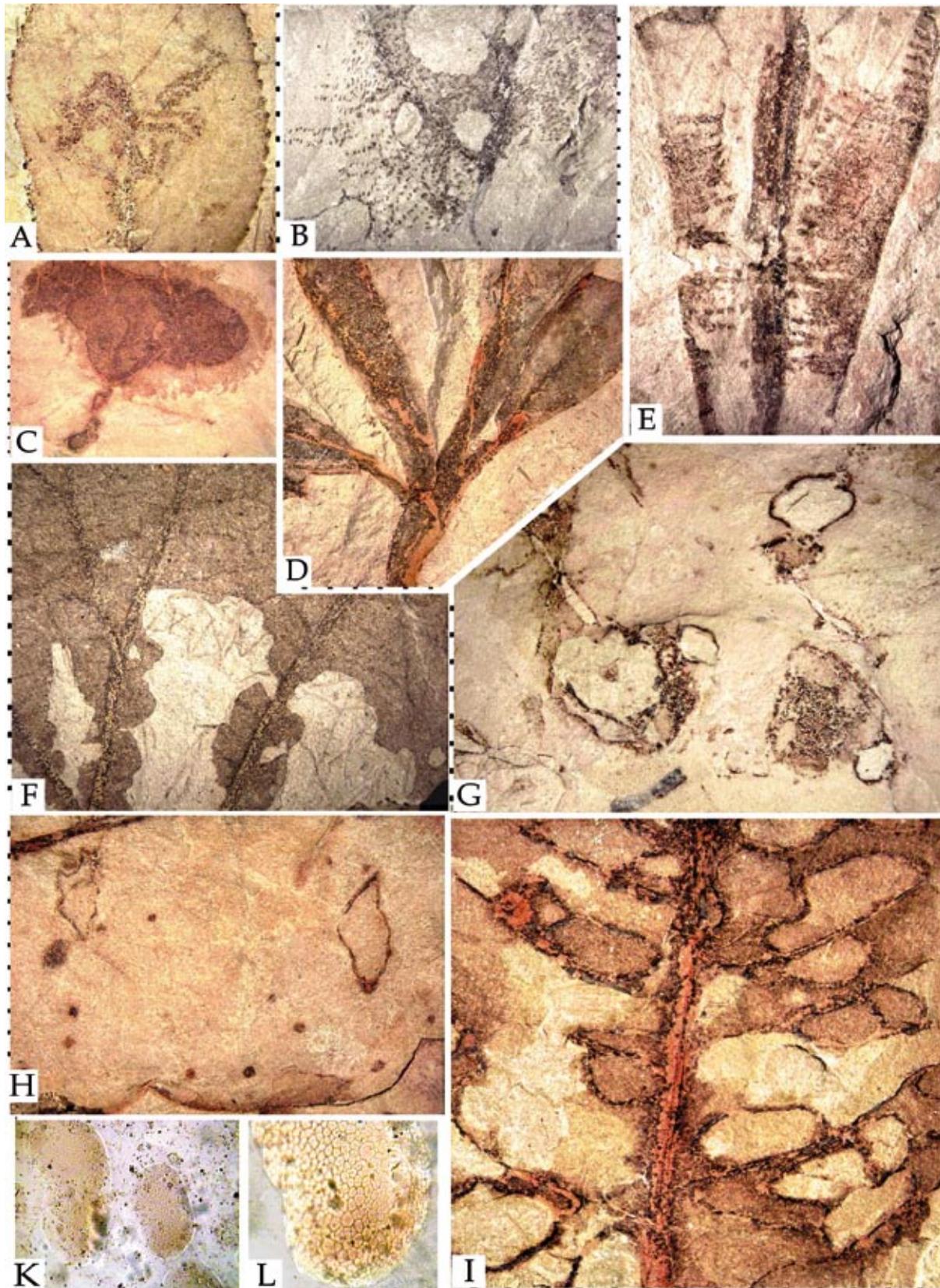


Figure 3. Insect traces on leaves from the Turonian of Gerofit (ruler point 1 mm). A. Ig1-138, digitate fly mine (dipterome) on *Platydebeya papilionacea*. B. IG1-1003, crenulate predation holes on dipterome on *Nelumbites arvensis*. C. IG1-588, deep reniform blotch mine with a fringe of shallow mining on *Platinervia integrifolia*. D. IG1-1003, linear mine spreading from petiole over the leaflet midribs of a stunted compound leaf *Dewalquea geroftica*. E. Ig1-539, dipterome with transverse frass rows over the whole leaflet of *Dewalquea geroftica*. F. IG1-739, incurvarioid case construction holes on *Eocercidiphyllites glandulosus*. G. Ig1-107, small blotch mines with cut-outs for case construction on *Nelumbites arvensis*. H. Egg scars and looping coleopteronomes on *Dewalquea geroftica*. I. IG1-644, a mosaic of deep and shallow lithocolletiform mines on *Dewalquea geroftica*. K, L. Ig1-239. Structurally preserved eggs with a network of intrachorionic meshes on *Eocercidiphyllites glandulosus*.

indicating a waterside habitat of the narrow-leaved (pro) angiosperms. Such egg set patterns are common in the Turonian as well, added by the midrib, marginal, transverse and scattered types, as well as by the egg masses or spumaria laid on leaves of waterside plants by the Odonata, as well as Hemiptera: Coccidoidea and Lepidoptera: Noctuidae. In the case of the larger scattered egg scars, a curculionid affinity (Coleoptera: Curculionidae) is prompted by the similar feeding marks that in this group are inflicted in much the same way as oviposition marks.

Procecidia

Outgrowths of plant tissue around insect eggs deposited on or into plant organs are sometimes called egg-galls, "Eiertaschen", pustular galls or procecidia (Ross 1932; Mani 1964). Examples from various groups of gall-makers show that cecidogenesis is a two-stage process starting with non-specific wound responses which alert the host plant defensive system. Procecidia expressed as bulges of leaf tissue around egg scar forming a more or less distinctive cupule are widespread in Gerofit assemblage (example in Fig. 2B) suggesting even greater than now significance of cecidogenous oviposition stage.

Galls

Cecidogenesis is a complex phenomenon the etiology of which includes abnormal histological and morphological developments, as well as specific enclosures for parasitic feeding and habitation. Their relative significance for the gall etiology is reflected in the classification of hystoid, organoid, and neoplasmatic (cataplasmatic or the more prominent protoplasmatic) galls (Küster 1911; Meyer 1987). A remarkable feature of the Cretaceous gall community is the paucity of prosoplasmatically expressive cecidia, represented by a scaly cephaloneon (Fig. 2E) comparable to some multichamber galls produced by the Aphidoidea, and by two types of small ceratoneons (Fig. 2D) similar to those of eriophyids, thrips, psyllids and other cecidozoa, but here tentatively ascribed to gall midges (Diptera: Cecidomyiidae) on account of their deciduousness. The microgalls are much more abundant (Figs 2C, H), some agglomerated into groups of large central and smaller peripheral lenticels as might have been produced by an aphid fundatrix and the gallicolae.

Organoid galls are most prominent in terms of their abundance, diversity, and morphological conspicuousness. The most widespread organoid effects are inflation of leaf petioles, webbing of petiolules in the palmate compound leaves, twisting, fasciculation, and splitting of stronger veins, as well as rolling of leaf margins (Figs 2F, I, K). In *Dewalquea gerofitica*, an extensive dissociation of vascular bundles (Fig. 2K) betrays a cecidomyiid gall-maker.

Mines

Mines of all major mining groups, Lepidoptera, Coleoptera, Hymenoptera, and Diptera, are represented in Gerofit mine assemblage. The functionally meaningful mine con-

figurations correspond to distinctive feeding habits, such as the mine-in-mine configurations resulting from two-stage mine development - a shallow early mine superimposed by the deeper late one (Figs 3C, D), characteristic of lithocolletiform mines (Lepidoptera: Gracilaridae). The coleopteronomes are recognized by their adherence to the stronger veins and by persistent egg remains, a rare feature in the other mining groups, except the Tischeriidae (Lepidoptera). The digitate mines (asteronomes) with double rows of frass pellets (Fig. 3A) are quite similar to those of extant Agromyzidae (Diptera). The whole-leaf mines with epidermal windows (Fig. 3E) are also assigned to dipteronomes (mainly of the Anthomyiidae). Mines with leaf pieces cut out for case construction are convincing evidence of temporary incurvarioid miners responsible for about 22% of all mine occurrences (Figs 3 F, G).

Irrespective of these provisional taxonomic assignments, the Cretaceous mine assemblage of Gerofit exhibit various habits of mine construction found, although in different proportions, in extant mine assemblages. Thus, blotches prevail over linear tracks, which is opposite to what is typical of the present day mining communities (Hering 1951, 1957). Another distinction is that the combined linear-blotch mine configurations (ophiostigmatonomes) are rare in the Cretaceous community, the linear segment being either negligibly short or altogether lacking, which means that larvae were indulged in blotch feeding immediately or soon after hatching.

A number of mine remains, in particular, the asteronomes, bear unequivocal evidence of mine predation (about 9% of all mine occurrences). Some mines are cut out as whole, the other are variously punctured (Fig. 3B), indicating a diversity of predators.

DISCUSSION

Our tentative taxonomic assignments of gall inducers suggest the leading role of cecidomyiids and aphids, with a much lesser cynipid contribution (Fig. 4). The Turonian cecidozoan community thus appears temperate (in particular, on account of aphid galls: Wool 1984) rather than tropical. However, the subordinate role of cynipids can also be explained by their later evolutionary development

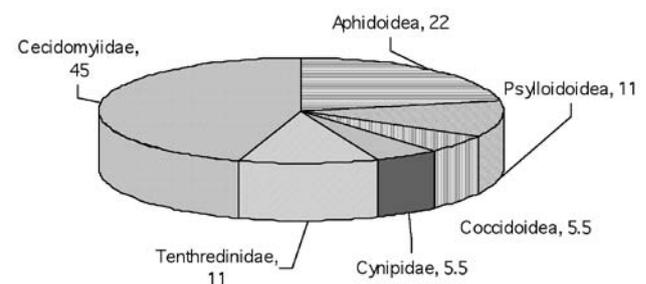


Figure 4. Relative contribution of various groups of cecidozoa based on tentative assignments of gall impressions in the Turonian of Gerofit.

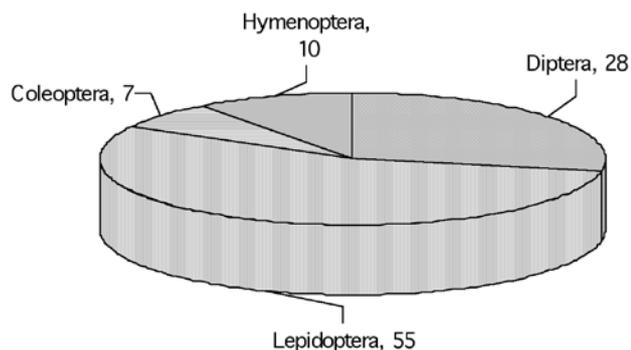


Figure 5. Relative contribution of various groups of mining insects based on tentative assignments of mine impressions in the Turonian of Gerofit.

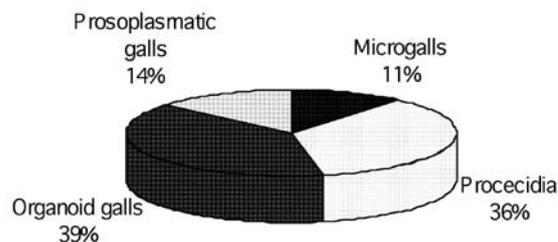


Figure 6. Relative significance of gall types in the Turonian of Gerofit.

as an advanced group of gall makers. About one half of the mines are the lepidopteronomes (Fig. 5), which is different from both temporal and tropical present day mining communities, the former being typically dominated by Diptera and the latter by Coleoptera (Hespenheide 1991).

From the functional point of view, cecidogenesis is considered as a co-adaptive interaction allowing host plants to localize the impacts of phytophagous parasites (Mani 1964, Ananthakrishnan 1984; Wool 1984). The prevalence of organoid effects over prosoplasmatic constructions in Gerofit assemblage (Fig. 6) thus signifies a low grade co-adaptive interaction in the early angiosperm – arthropod ecosystems. Species-specificity of cecidogenous effects is commonly held as an advanced feature of such systems, and it is scarcely a co-incidence that the only prosoplasmatically advanced *Cephaloneon* form (Fig. 2E) is also the one of a few strictly monophagous galls. At the same time, the most destructive organoid form, the midrib splitting gall of *Dewalquea gerofitica* (Fig. 2K), is also monophagous, or nearly so (with a few occurrences on *Eodebeya angusta*, a co-dominant species of mangrove community).

Since blotches are typically more damaging for leaf photosynthesis than linear tracks, a high ratio of blotch to linear mine occurrences in the Cretaceous trace community (67% and 22% respectively) is evidence of a low co-adaptive status in the same way as the prevalence of organoid galls (above). In addition, its relatively low evolutionary advancement level is indicated by a number of positive and negative features, such as:

- (1) The occurrence of whole leaf area – whole depth mines, some with epidermal windows (5%);
- (2) The incidents of petiole to midrib (primary vein) mining causing leaf stunting and deformity (Fig. 3D);
- (3) The rarity of frass-less mines representative of epidermal sap feeders, in conjunction with:
- (4) The rarity of serpentine tracks (1%), the most parsimonious mine construction in terms of the track length/area ratios.

With exclusion of occasional one-time occurrences, few mine types, in particular those of temporary miners leaving traces of case construction (Figs 3F, G) are monophagous.

Predation traces are more frequent on the leaf mines of the broadleaved *Eocercidiphyllum – Platanervia – Platydebeya* plant assemblage than on those of the narrow leaved coastal (mangrove) *Dewalquea – Eodebeya* assemblage. Negative correlation of predation frequencies and the overall mine occurrences testify to the top down regulation *vis-à-vis* the bottom up regulation by the plant host resistance. Their relative significance affects, albeit indirectly, the dimensions, morphology, topography and on leaf co-occurrence of different mine types.

Co-occurrence of two or more mines on leaf is quite common in Gerofit assemblage as a whole, being more frequent in the coastal assemblage than in the inland broadleaved assemblage. This feature is regulated in a complex way, the crowded mines being more conspicuous for predators than solitary mines, but association of different mine types being at the same time protective for a specifically targeted mine type. The plant host resistance either increases or decreases with crowding, in the latter case, known as “weakness parasitism”, encouraging more parasites to join.

Temporary mining (through early development, after which the larva escapes to the surface) is likewise a complexly regulated trait, the top down component of which confers selection for smaller size – shorter mining period (Hering 1951). In Gerofit assemblage, traces of temporal mining followed by case construction occur either on leaves of a typical deciduous aspect, such as *Eocercidiphyllites glandulosus* and *Platanervia integrifolia*, or on emergent leaves of aquatic angiosperm, *Nelumbites arvensis*, thus indicating the leading role of plant ecology (deciduousness, susceptibility to flooding) in regulation of the trait. However, the relative frequencies of temporary mining traces in the inland and coastal assemblages are correlated with the frequencies of predation marks, indicating a contribution of top down regulation. Although paleoclimatic inference based on modern analogies are inevitably biased

by distinctions brought about by evolutionary, rather than climatic, developments, such criteria as the relative abundance of temporary miners seem instructive, being directly correlated with climate seasonality and deciduousness.

CONCLUSIONS

Our data show explosive evolution of leaf parasitism after a preparatory stage, in which the preexisting groups of parasitic arthropods have transferred from the declining gymnosperms to the rising angiosperms. Great morphological plasticity of latter might enhance diversification of parasitic habits. At the same time, such features of Cretaceous trace assemblages as the abundance of procecidia and the prevalence of devastative habits among the miners and gall makers indicate a relatively low level of co-evolutionary advancement. Traces of gall and mine predation are evidence of incipient top down regulation over the plant – arthropod communities (Labandeira 2002) superimposed upon the bottom up regulation by plant defense, jointly contributing to evolutionary advancement of the system. Indirect evidence of mine/gall abundances, their on leaf co-occurrence and the temporary mining habits reveal different advancement levels for the contemporaneous coastal (mangrove) and inland communities.

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REFERENCES

- ANANTHAKRISHNAN, T.N. 1984. Adaptive strategies in cecidogenous insects. In: *The Biology of Gall Insects*, ANANTHAKRISHNAN, T.N. (Ed.). Arnold, Baltimore: 1-10.
- DOBRUSKINA, I.A., PONOMARENKO, A.G. & RASNITSYN, A.P. 1997. Fossil insect finds in Israel. *Paleontological Journal (Moscow)*, 31: 91-95.
- HELLMUND, M. & HELLMUND, W. 1996. Zur endophytischen Eiablage fossiler Kleinlibellen (Insecta, Odonata, Zygoptera), mit Beschreibung eines neuen Gelegetyps. *Mitteilungen Bayerische Staatssammlung für Paläontologie und historische Geologie*, 36: 107-115.
- HERING, E.M. 1951. *Biology of the leaf miners*. W. Junk, The Hague: 420 pp.
- HERING, E.M. 1957. *Bestimmungstabellen der Blatminen von Europa*, Bd. III. W. Junk, The Hague: 86 pp.
- HESPENHEIDE, H.A. 1991. Bionomics of leaf-mining insects. *Annual Review of Entomology*, 36: 535-536.
- HINTON, H.E. 1981. *Biology of insect eggs*. Pergamon Press, Oxford: 1125 pp.
- KRASSILOV, V.A. 2007. Mines and galls on fossil leaves from the Late Cretaceous of southern Negev, Israel. *African Invertebrates*, 48: 13-22.
- KRASSILOV, V.A. & BACCHIA, F. 2000. Cenomanian florule of Nammoura, Lebanon. *Cretaceous Research*, 21: 785-799.
- KRASSILOV, V.A., LEWY, Z., NEVO, E. & SILANTIEVA, N. 2005. Late Cretaceous (Turonian) Flora of southern Negev, Israel. *Pensoft, Sophia*: 252 pp.
- KÜSTER, E., 1911. *Die Gallen der Pflanzen*. S. Hirzel, Leipzig: 437 pp.
- LABANDEIRA, C. 2002. Paleobiology of predators, parasitoids, and parasites: accommodation and death in the fossil record of terrestrial invertebrates. *The Paleontological Society Papers*, 8: 211-249.
- MANI, M.S. 1964. *Ecology of Plant Galls*. W. Junk, The Hague: 434 pp.
- MEYER, J. 1987. *Plant Galls and Gall Inducers*. Borntraeger, Berlin: 201 pp.
- ROSS, H. 1932. *Practicum der Gallenkunde*. Springer, Berlin: 211 pp.
- SILANTIEVA, N. & KRASSILOV, V. 2006. Evolution of Early Angiosperm Ecosystems: Albian - Turonian of Negev, Israel. In: *Mesozoic Terrestrial Ecosystems and Biota*, P.M. BARRETT P.M. & EVANS, S.E. (Eds.), Natural History Museum, London: 118-122.
- WOOL, D. 1984. Gall-forming aphids. In: *The biology of gall insects*, ANANTHAKRISHNAN, T.N. (Ed.). Arnold, Baltimore: 11-58.

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