

GENERAL BIOLOGY

Pollen in Guts of Fossil Insects as Evidence for Coevolution

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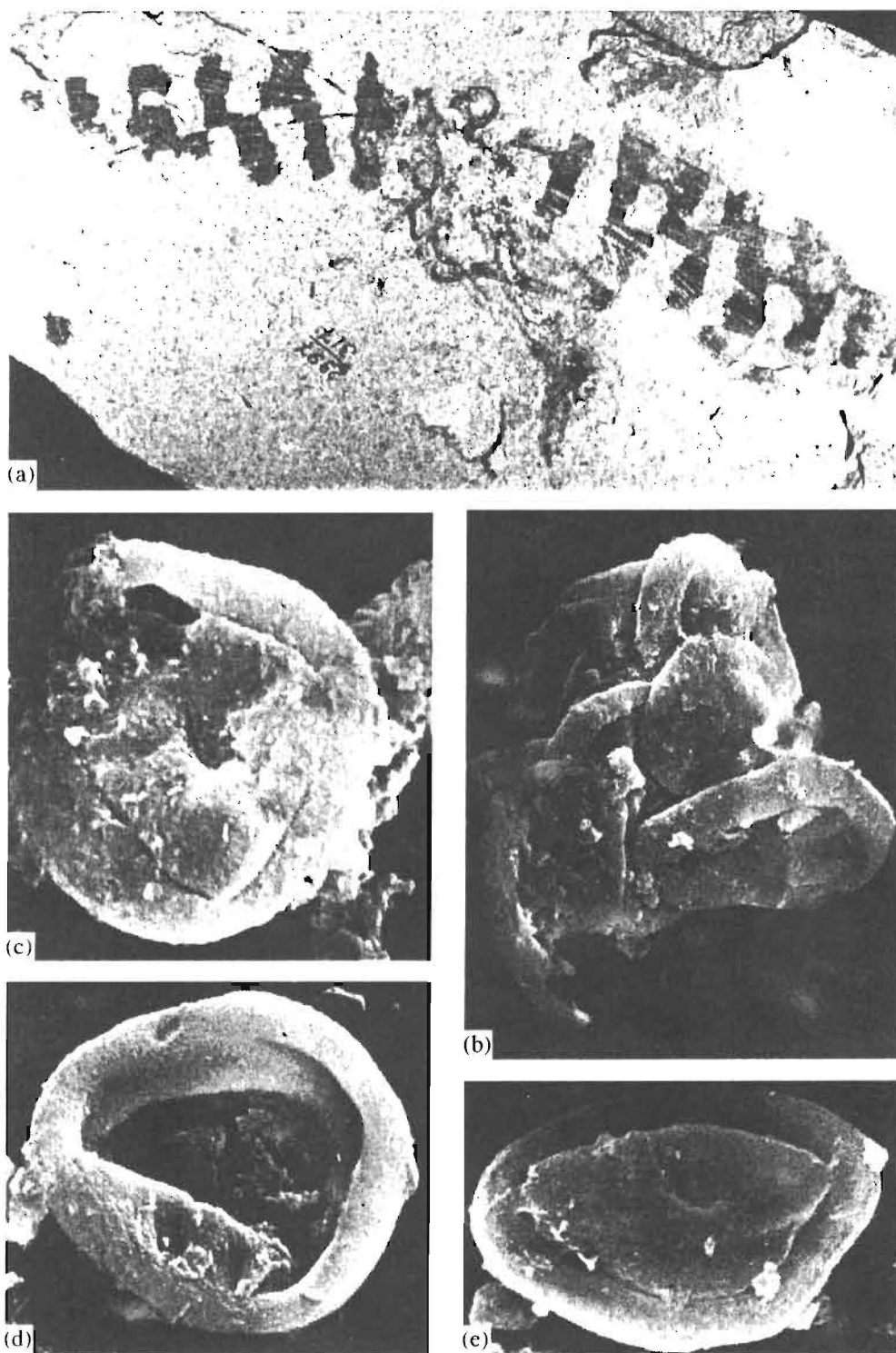
It has long been assumed that interaction between insects and plants is an important factor of their evolution. However, only recent paleontological evidence has provided factual data for the study of coevolution. We developed a method to obtain plant remains from guts of fossil insects through maceration of their contents and subsequent examination under an electron microscope. Plant objects obtained by this method, e.g., leaf cuticles and pollen grains, are sometimes completely intact, which allows us to study microstructural characteristics that cannot be studied by other methods. The data obtained can be used for reconstruction of the functional structure of ecosystems that existed in past geological epochs, as well as feeding patterns and plant consumption in certain groups of extinct insects. These data allow the elements of the spatial structure of plant communities to be reconstructed.

The experimental material was obtained from the large collection of arthropods at the Institute of Paleontology, Russian Academy of Sciences, which comprises several thousands of specimens from deposits dated back to the Carboniferous Period through the Pleistocene. We were the first to demonstrate that pallinophagia was characteristic of several groups of extinct insects that lived in the Permian and early Cretaceous Periods. We isolated saccate (mostly protosaccate) pollen from guts of early Cretaceous Xyelidae that lived in the Trans-Baikal region [7]. The main food of three insect species found in Permian deposits in Chekarda (the Cis-Ural region) was the striated pollen of Glossopteridales, Peltaspermales, and primitive conifers characteristic of Permian and Triassic deposits. On the basis of these data, we hypothesize that the convergent formation and the parallel development of striated morphotypes of large protosaccate pollen and the corresponding changes in the female reproductive system of gymnosperms in Subangaraland and Gondwanaland were related to development of insect pollination.

We studied fossil insects from Mikhailovka (Aulie, the Karatau Range, southern Kazakhstan), an area in which numerous examples of upper Jurassic flora and fauna have been found [2, 6]. Pollen of *Classopollis* was found in the guts of orthopterans from two *Aboilus* species. Genus *Aboilus* was a primitive representative of family Prophalangopsidae (Ensifera, Orthoptera) and was widespread in the Karatau oryctocenosis. To date, 12 species from this genus have been found in Karatau and described [3]. They differ considerably in the structure of the male stridulation apparatus; females can be easily classified according to the size and patterning of forewings. The examined specimens, 2904/1675 and 2997/317, were well-preserved remains of females. One of them (IP, no. 2904/1675b) was classified as *A. ampulus* Gorochov. The other (IP, no. 2904/1675) resembled *A. dilutus* Gorochov with respect to some characteristics of the wing (three incomplete, dark, transverse bands in the distal third and many small spots throughout the remaining part of the wing); however, the wings differed from those of *A. dilutus* in some details of the pattern and were considerably longer (the forewing was 65 mm long, versus 48.5 mm in *A. dilutus*). This genus belongs to a family that was abundant during the late Mesozoic Era and has been preserved to date only as a relict group comprising few species. Subfamily Aboilinae, which was abundant during the Middle Jurassic through the Early Cretaceous Epochs in Eurasia from central Europe to Japan, is completely extinct. Aboilinae were ancestors of modern grasshoppers (Tettigoniidae) and resembled them in appearance [4]. Judging from their habitus, they apparently lived in shrubs and woods [8].

Until the present, grasshoppers have not been assigned to pollinivores, although certain studies [5] revealed that pollen constituted 10 to 100% of the food contained in crops and intestines of 80 out of 100 individuals from different subfamilies. The fact that pollinivory is widespread suggests that it is an old, primitive trophic adaptation in grasshoppers. Our data confirm this suggestion.

Almost the entire contents of the guts of both fossil insects consisted of *Classopollis* pollen (figure) aggregated into large lumps. Pollen grains were flattened,



Pollen isolated from the gut of a fossil insect. (a) *Aboilus ampulus* Gorochov from upper Jurassic deposits in Karatau (IP, specimen no. 2997/317) with pollen in the gut ($\times 1$); (b) a lump of pollen grains of *Classopollis* (scanning microscope, $\times 1700$); (c–e) individual pollen grains at the proximal and distal positions (scanning microscope, $\times 3000$).

ellipsoidal, almost disk-shaped, and were at different stages of ripeness. Mature grains were 22–24 μm (up to 25.5 μm) long, and immature ones, 15–18 μm long at the longer axis. An equatorial girdle, 3.5 μm wide,

encircled the body of grain as a circular sac and was separated from it with a rimula. The proximal tetrad mark was triangular and, in some pollen grains, covered with a membrane containing small granules scat-

tered over it. This portion of grain still showed exine filaments, which kept the grains together in the tetrad. The distal pore-like structure had a membrane penetrated by fine micropores. The covering layer of the outer wall of grain was disturbed in places and revealed an infrastructure consisting of club- and cone-shaped rods that fused together to form irregular cellules. The structure of these grains was typical for *Classopollis* [10]; however, the porous membrane of the distal aperture and the granular membrane of the tetrad mark were observed for the first time. We also demonstrated that elements usually assigned to the grain sculpture were actually elements of grain infrastructure, which was located under a thin tectum and observable with an electron microscope. These data suggest that determination of species of genus *Classopollis* should be revised. The pollen grains from guts of *Aboilus* resembled the dispersed grains of *C. bussonii* Reyre and *C. noelii* Reyre [9].

The importance of this finding is primarily related to the fact that *Classopollis*, the pollen-grain morphotype with angiosperm features abundant during the Mesozoic Era, has become an object of phytostratigraphic, paleoclimatic, and phylogenetic studies [1]. The pollen of *Classopollis* was the first pollen to have the pore and zonal apertures, the striated equatorial zone, and the columellate infrastructure characteristic of angiosperms and functionally related to the mechanism of reproductive self-incompatibility; it emerged at the time when the single-grooved, distal-grooved morphotype characteristic of gymnosperms was completely prevalent. This pollen was found in sporangia, which demonstrated that *Classopollis* belonged to Hirmerellaceae ("Cheirolepidaceae"), an extinct group which is routinely classified as a conifer. However, the data that one of the authors obtained on the structure of female strobili, as well as pollinological traits, allow us to classify them as a separate branch of Gnetophyta. As a result, our view on the Mesozoic vegetation changes substantially. Along with conifers, Ginkgophyta, and Cycadophyta, scale-leaved Gnetophyta was also one of the prevalent groups. The fossils assigned to this group are masses of separated leaves, shed small twigs (as in ephedra), and cupules with wing-shaped processes, which served for seed dissemination. The pollen apparently germinated on the surface of cupules, without penetrating into the pollen chamber.

During the Jurassic Period, Hirmerellaceae were abundant from the equator to 50° north latitude, with virtually none of them penetrating to the zone of *Phoenicopsis* deciduous forests. The common notion that *Classopollis* is an indicator of an arid or semiarid climate does not agree with taphonomical data. The pollen is most abundant in coastal deposits, where it constitutes as much as 80% of palynological spectra. Findings of large insects with well-preserved gut contents in Karatau marls indicate that their habitats were close to the coastline; this agrees well with the abundance of remains of Hirmerellaceae, whose trunks are often found in the same positions as they had when the plants were alive. These data indicate that the

xeromorphy of Hirmerellaceae, which is usually attributed to a dry climate, was actually determined by their belonging to littoral communities of sea coasts and carbonate lakes characterized by adaptation to physiologic dryness. Harmomegathy structures of their pollen were related to insect pollination rather than to the climate.

Our evidence that insects ate *Classopollis* pollen agree with the high pollen productivity of Hirmerellaceae and the data (obtained from reconstructions) that they were a dominant component of coastal formations. Large orthopterans flying small distances transferred much pollen, which was in the air for a long time. This allows us to explain such characteristic features of *Classopollis* pollen as dispersal in tetrads and reduction of the equatorial sac, which was transformed into a harmomegathy girdle regulating the volume of the pollen grain. In addition, it was necessary to protect developing ovules, to develop a closed cupular ovule covering, and to switch to extraovular germination of pollen. Therefore, the evolution of Hirmerellaceae resulted in development of the traits of angiosperms. This direction of the phylogeny of Mesozoic plants was a result of coevolution and was sustained by interaction with insects, involving different components of pioneering communities.

Thus, we have obtained the first conclusive evidence for the participation of insects in the formation of large groups of higher plants and the development of the main characteristic features of angiosperms.

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