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RUTACEAE-FEEDING *AGONOPTERIX* HÜBNER (LEPIDOPTERA: ELACHISTIDAE) IN ILLINOIS

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Abstract.—The most recent taxonomic treatment of North American moths of the genus *Agonopterix* Hübner (Lepidoptera: Gelechioidea: Elachistidae) recognized two Rutaceae-feeding species, *Agonopterix nigrinotella* (Busck) and *Agonopterix pteleae* Barnes and Busck. Ecological, morphological, and molecular data presented in this paper indicate that four species of *Agonopterix* feed as larvae on Rutaceae in Illinois. *Agonopterix costimacula* Clarke (**revised status**) and *A. pteleae* feed on hoptree, *Ptelea trifoliata* (Linnaeus). Both of these species are multivoltine. *Agonopterix nigrinotella* and a previously unrecognized species, described here as ***Agonopterix paulae* Harrison, n. sp.**, feed on prickly-ash, *Zanthoxylum americanum* Miller. *Agonopterix nigrinotella* is univoltine, whereas *A. paulae* is bivoltine. Larval feeding tests determined that each of the four species is strictly monophagous and that literature reports of *A. nigrinotella* on *Carya* sp. and *Agonopterix argillacea* (Walsingham) on *Ptelea trifoliata* are apparently erroneous. Diagnoses and dichotomous keys are given for identifying mature larvae and adults of the four Rutaceae-feeding species.

Key Words: Microlepidoptera, Gelechioidea, new species, voltinism, food plant specificity, Rutaceae, *Zanthoxylum, Ptelea,* mitochondrial DNA, *Cytochrome Oxidase II,* North America, Illinois

Although the North American component of the genus *Agonopterix* Hübner has been studied more extensively than have most other "microlepidoptera" groups on this continent (Clarke 1941, Hodges 1974, Berenbaum and Passoa 1999), and larval food plants are known for almost all species, complete life histories have been evaluated for only a few (Hodges 1974). Because of such inadequate life history information, the complex of Nearctic Rutaceae-feeding species centered around *Agonopterix nigrinotella* (Busck) represents an element of the genus that has remained unresolved.

The earliest description of a Nearctic Rutaceae-feeding *Agonopterix* species was that of *A. nigrinotella* (Busck 1908), which was described as *Depressaria nigrinotella.* Subsequently, Barnes and Busck (1920) described *Agonopteryx* [*sic*] *pteleae,* and Clarke (1941) described *Agonopterix costimacula.* Clarke proposed that *A. pteleae* and *A. costimacula* feed as larvae only on *Ptelea trifoliata* (L.), and that *A. nigrinotella* feeds only on *Zanthoxylum americanum* Miller.

Hodges (1974) recognized *A. pteleae* as a distinct species but relegated all other Rutaceae-feeding *Agonopterix* to *A. nigrinotella* (with *A. costimacula* sunk as a junior synonym of *A. nigrinotella*). Hodges acknowledged that more than one genital type

was present in the moths ascribed by him to *A. nigrinotella.* His justification for retaining all of these entities under one species name was that a single genital type could be found in moths representing more than one of the color forms that he recognized, and that males of a single genital type had been reared from both *Z. americanum* and *P. trifoliata.*

Field investigations conducted by S. Passoa in the 1980s revealed a diverse fauna of Rutaceae-feeding *Agonopterix* in Illinois, with larvae utilizing both of the two native rutaceous plants found in the state, *Ptelea trifoliata* (Linnaeus) and *Zanthoxylum americanum* Miller. The apparent presence of all of the relevant moth and plant species in this same geographic area suggested that a study of Rutaceae-feeding *Agonopterix* in Illinois could provide a clear resolution to species identities within this problematic group; presented herein are the results of such a study.

Materials and Methods

For rearing and for food plant specificity trials, larvae were placed individually in 30 ml plastic snap-lid cups containing a small amount of leaf material. In food plant specificity trials, first-stadium larvae were subjected to no-choice feeding tests on plants other than the known field hosts: *A. pteleae* and *A. costimacula* on *Z. americanum, A. paulae* and *A. argillacea* on *P. trifoliata,* and *A. nigrinotella* on both *P. trifoliata* and on two species of *Carya* Nuttall, the latter genus having been recorded as a food plant by Hodges (1974). After 24 h, each larva was placed in a new cup containing the field host and was reared to adult, to confirm its identity and its normal state of health.

Larvae to be preserved for morphological study were placed for 24 h in XAA solution (10% xylene, 80% absolute ethanol, 10% glacial acetic acid, saturated with distilled water) and then transferred to 70% ethanol, 30% distilled water for permanent storage. Drawings of larvae, as well as all other drawings, were done through a Wild M5 microscope equipped with a Wild M5 Zeichentubus drawing tube, at magnifications of 25× to 40×; larvae were submerged in 70% ethanol in a clear glass depression slide. Names of primary setae follow those given by Stehr (1987).

For study of genitalia, the entire abdomen of each dried specimen was pried off with forceps. The abdomen was macerated in 20% potassium hydroxide in distilled water for eight to 24 h, and then dissected in 70% ethanol. Iridectomy scissors were used to make a longitudinal slit in the lateral membrane, beginning at the anterior end of the abdomen and running posterior to the hind margin of the 7th (females) or 8th (males) abdominal segment. Genitalia were stained overnight in 63% ethanol, 27% distilled water, and 10% mercurochrome. While in the stain, genitalia were positioned for viewing in ventral aspect and were kept in this position with glass chips laid over them. After staining, they were cleaned with a 00000 gauge sable brush in 70% ethanol; from here, they were either placed directly into a vial of 70% ethanol for storage or were immersed briefly in clove oil and then mounted in Canada balsam on microscope slides.

For genetic analysis, adult moths were frozen alive, and genomic DNA was extracted following the protocol of Sperling et al. (1994). A 522-bp fragment of mitochondrial *Cytochrome Oxidase II* (*COII*), homologous with mitochondrial genome positions 1691 through 2212 of *Yponomeuta malinellus* Zeller (Sperling et al. 1995), was amplified by polymerase chain reaction (PCR) (Saiki et al. 1985, 1988) using primers C2-J-3138 (5′ AGCGCCTCTCCTTTAATAGAACA 3′) and C2-N-3661 (5′ CCACAAATTTCTGAACATTGACCA 3′) (Simon et al. 1994). Each PCR sample was sequenced for its entire length in both 5′–3′ and 3′–5′ directions, by using the opposing terminal primers; sequencing was done in the University of Illinois Automated Sequencing Laboratory. One individual

of each species was sequenced, with the exception that partial sequence was obtained from a second individual of *A. costimacula.*

Abbreviations of Institution and Collection Names.—USNM: National Museum of Natural History, Smithsonian Institution, Washington, D.C., USA; INHS: Illinois Natural History Survey, Champaign, Illinois, USA; JRW: Collection of James R. Wiker, Athens, Illinois, USA; SCP: Collection of Steven C. Passoa, Columbus, Ohio USA.

Results and Discussion

One of the insects involved in the present study is a described species, *A. costimacula.* This species is currently placed as a junior synonym of *A. nigrinotella.*

Agonopterix costimacula Clarke, 1941, **revised status**

The original description and type material are as designated by Clarke (1941). In this paper, we provide ecological, morphological, and molecular evidence to support the conclusion that *A. costimacula* is a valid species.

One of the insects in this study proved to be a previously undescribed species. This species did not correspond to type material of the three Nearctic Rutaceae-feeding *Agonopterix* species mentioned above, nor with *Agonopterix argillacea* (Walsingham) or *Apachea barberella* (Busck), two additional Nearctic Depressariinae species that have been recorded from Rutaceae (Hodges 1974).

Likewise, we determined that the novel species does not represent an introduced population of one of the three European species of Rutaceae-feeding *Agonopterix, A. rutana* (Fabricius), *A. furvella* (Treitschke), and *A. pupillana* (Wocke), nor of either of two additional Rutaceae-feeding Depressariinae of Europe, *Depressaria ruticola* (Christoph) and *Horridopalpus dictamnella* (Treitschke). Larvae, adult moths, and genital morphology of the European species were illustrated variously by Stainton (1861, 1870), Rebel (1899), and Hannemann (1953). The following is a description of the novel insect.

Agonopterix paulae **Harrison, new species**

(Figs. 1A, 2A, 3A, 4A, 5A)

Adult (Fig. 1A).—Forewing length, 10.0–10.5 mm. *Antenna:* Pedicel dark brown, ringed with ochreous in apical one-fourth; flagellum shining gray brown, slightly paler ventrally than dorsally. *Labial palpus:* Basal segment light brown with blackish-brown lateral patch; second segment 6× as long as first, clear light brown medially, light brown flecked with dark brown laterally, numerous dark-brown scales ventrally where scales assume a fan-like arrangement, giving segment a dilated appearance; third segment smoothly scaled, acuminate, nearly as long as second, ochreous brown with dark-brown bands at one-sixth and two-thirds length, and finely tipped with dark brown at extreme apex. *Maxillary palpus:* Minute, folded over base of haustellum, whitish brown. Haustellum clothed in whitish-brown scales. *Face:* Smoothly scaled, shining whitish brown, with a small number of blackish-brown scales near compound eye. *Head:* Roughly scaled, ochreous with medial brown line; occipital scale band pale ochreous medially, brown laterally. *Thorax:* Terga and tegula ochreous overlaid with brown; a pair of whitish-ochreous tufts on metathoracic tergum. *Proleg:* Coxa whitish ochreous, dusted with brown except at apex; lateral surface pearl white with a few brown scales; femur whitish ochreous, heavily dusted with brown medially, with a concentrated brown blotch laterally; tibia slightly shorter than femur, with somewhat dilated appearance due to loose scaling, clear pale ochreous laterally, pale ochreous dusted heavily with brown medially; tarsus equal in length to tibia, with basal tarsomere 3× as long as each of remaining four, brown, apices of basal two tarsomeres and entire apical tarsomere ochreous. *Mesoleg:* Coxa pale

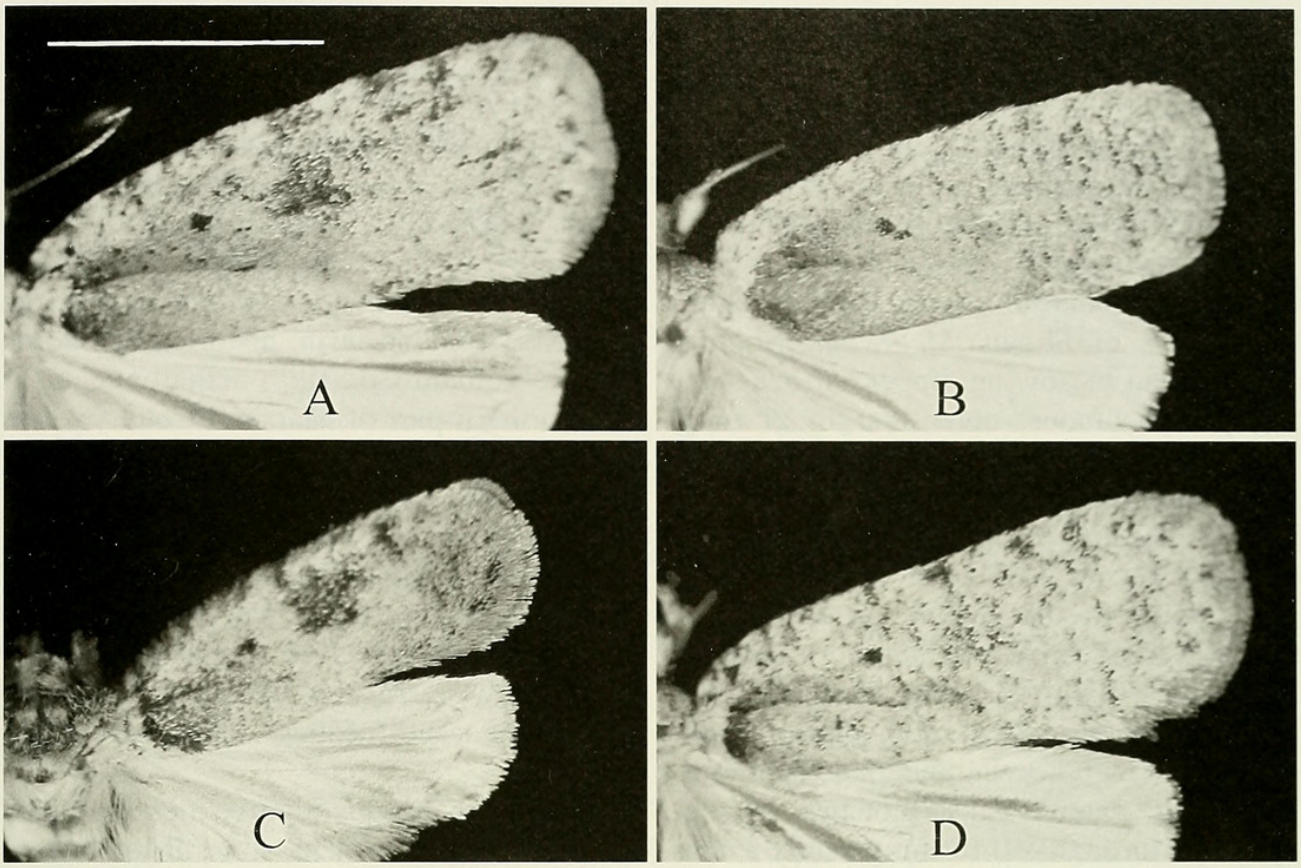


Fig. 1. Right mesothoracic wings of Nearctic Rutaceae-feeding *Agonopterix*; dorsal aspect. A, *A. paulae*. B, *A. nigrinotella*. C, *A. pteleae*. D, *A. costimacula*. Scale = 5.0 mm.

ochreous with central brown patch, scales at apex overlying region of trochanter; femur pale ochreous dusted with brown; tibia subequal to femur in length, scales arranged to give a dilated appearance, pale ochreous with a brown lateral blotch near base and a brown band at three-fourths length; a pair of apical spurs, both brown, medial one 3× as long as lateral; tarsus equal to tibia in length, basal tarsomere as long as remaining four combined, brown, apices of all tarsomeres and entire apical tarsomere ochreous. *Metaleg:* Coxa and femur as for mesoleg; tibia slightly longer than femur, with loose scaling giving dilated appearance, almost entirely pale ochreous, with a few scattered brown scales; two pairs of spurs, at approximately one-half tibia length and at apex, shining ochreous, medial one 3× as long as lateral in both pairs; tarsus almost as long as tibia, basal tarsomere slightly longer than remaining four combined, ochreous medially, brown with apex of each tarsomere ochreous laterally. *Mesowing:* Length, 9.8 mm. Upper surface ground color medium ochreous brown; a small, blackish-brown patch at base of costa, base of wing otherwise ochreous to one-tenth length, then met by a narrow band of blackish brown, this band broken above fold by an outward projection of basal ochreous color; only a slight diffusion of dark-brown scales extends apically from blackish-brown band; a small, blackish-brown spot on fold, a second, similar spot almost directly anterior to it, and a third such spot anterior and basal to second; six or seven blackish-brown strigils on costa; each of two most prominent of these, at four-fifths wing length, with a similar blackish-brown mark immediately below it; a grayish-brown blotch sits over end of cell, this blotch not reaching costa; at its posteroapical margin containing a small but distinct whitish spot; veins R_4, R_5, and M_1 delineated apically by blackish-brown streaks;

scaling becoming slightly paler than ground in apical one-eighth of wing; outer edge of wing membrane marked by four or five small, blackish-brown spots; fringe concolorous with ground, with no discernible lines; underside shining grayish ochreous, anterior one-sixth ochreous with occasional dark strigils, extreme apex ochreous with fine points of brown. *Metawing:* Slightly shorter than mesowing; upper surface shining pale ochreous, outer margin of wing membrane finely scaled with brown; fringe pale ochreous basally, darker ochreous apically, with faint, incomplete lines near outer margin of wing membrane and near fringe apex; underside pale ochreous, mottled irregularly with brown in anterior one-third and around margin of wing membrane. *Abdomen:* Upper surface shining pale ochreous dusted with brown; underside ochreous, with large, lateral black blotches on segments III through VII forming nearly a continuous line; small, medial, paired black spots on segments IV through VII; a pair of lateral black dashes on terminal segments.

Larva (Fig. 2A).—Neonate yellow with dark-brown head capsule; larvae of subsequent stadia pale green with roughly concolorous head capsule; area of stemmata black in all stadia; in second and subsequent stadia, a black blotch appears on lateral surface of prothorax, and in third and subsequent stadia a black lateral stripe appears on anal shield. Length of mature larva, 18.5 mm (n = 5, range 18.1–19.2 mm).

Biology.—The larval food plant is *Z. americanum.* Eggs are laid either singly or in small masses. In the latter case, larvae initially feed communally between two leaflets that they have silked together, and their feeding at this stage creates a distinctive pattern of damage in which the affected leaflets assume a singed appearance. While still in the first stadium, the larvae leave the communal leaflets and establish themselves individually; from this point on, they live in the rolled margins of leaflets. There are evidently two generations per year, with mature larvae in central Illinois occurring in late June/early July, and again in late July.

Geographic range.—*Agonopterix paulae* has been recorded from Illinois counties Piatt, Jo Daviess, Vermilion, Coles, Menard, and Marshall (the known populations of *Z. americanum* and *A. paulae* on the former M. O. Glenn farm, historically recorded as "Putnam Co., Ill.", actually occur in the northern part of Marshall County). The insect may occur over the entire eastern USA, wherever the food plant is found, as was suggested for *A. nigrinotella* by Clarke (1941). The status of this species on native Rutaceae species that occur in areas other than the Midwest is not presently known, as is likewise true for the other three Nearctic Rutaceae-feeding *Agonopterix* species.

Diagnosis (for quantitative measurements of some of the character states listed below, consult the keys to species).—The mature larva of *A. paulae* is unique among the Rutaceae-feeding species in having black markings on the thorax and anal shield. *Agonopterix nigrinotella* lacks these markings but has prominent pinacula at the bases of the D setae on most of the body segments, whereas *A. pteleae* and *A. costimacula* are pale green with no black markings or pinacula on the body (a blackish marking on the genal area of the head may in all four species display individual variation from absent to prominent). The adult of *A. paulae* can be distinguished from *A. nigrinotella* by the much less uniform coloration of the mesowing, and from *A. pteleae* by the absence of upturned scales on the dorsal surfaces of the mesowings and thorax. It can be separated from *A. costimacula,* which it most closely resembles, by the relatively dark, ochreous-brown ground color, presence of a diffuse, blackish-brown area surrounding the pale spot on the cell in the mesowing, and presence of several blackish, longitudinal vein-streaks in the outer part of the forewing of *A. paulae* (*A. costimacula* with relatively pale, clay-brown

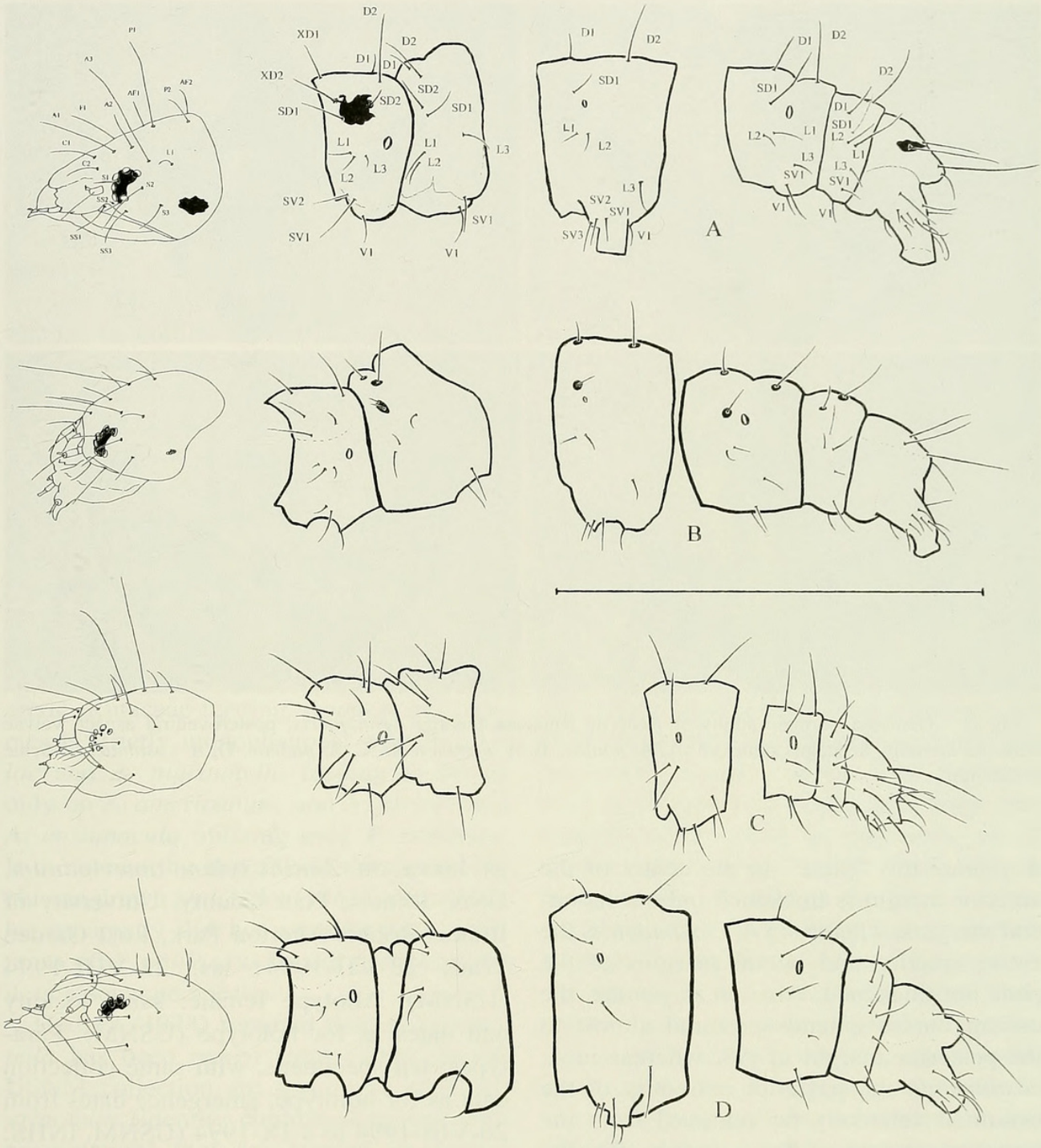


Fig. 2. Mature larvae of Nearctic Rutaceae-feeding *Agonopterix*; left lateral aspect. A, *A. paulae*. B, *A. nigrinotella*. C, *A. pteleae*. D, *A. costimacula*. Segments illustrated are, from left to right, head, first and second thoracic, first abdominal, and eighth through tenth abdominal. Scale = 5.0 mm.

ground color, with no dark area other than a narrow ring surrounding the spot on the cell, and no vein-streaks). In the male genitalia of *A. paulae* (Fig. 3A), the clasper is much straighter (less curved), and the valve is relatively longer and narrower, and more sharply curved dorsad at about half its length, than in the other three species. The juxta is quadrate and similar to that of *A. nigrinotella* and *A. costimacula*. In the female genitalia (Figs. 4A, 5A), the anterior margin of A8 is broadly squared (narrowly squared in *A. nigrinotella*, Figs. 4B, 5B, and broadly rounded in *A. pteleae*, Figs. 4C, 5C). Overall, *A. paulae* is similar to *A. costimacula* (Figs. 4D, 5D), except that in

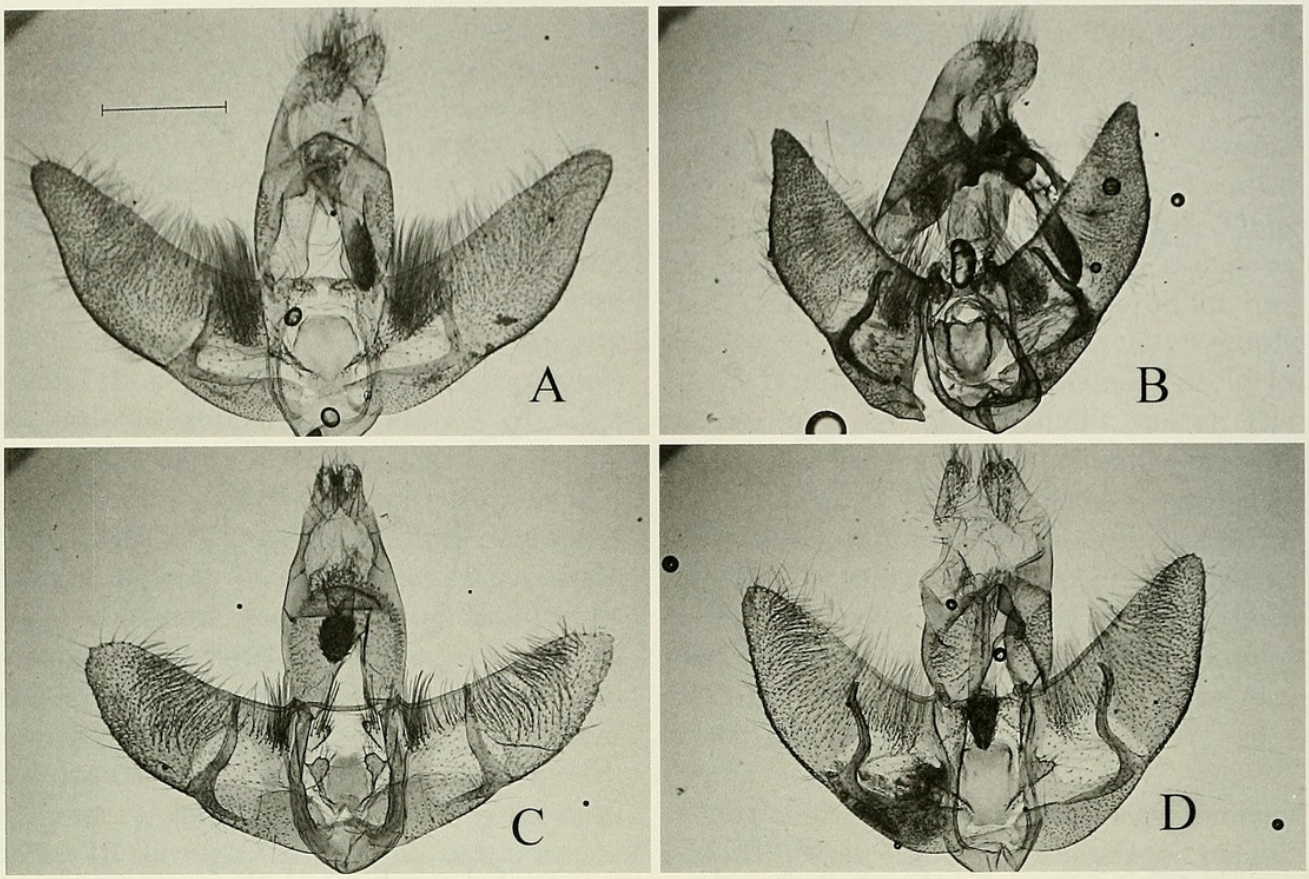


Fig. 3. Genitalia of male adults of Nearctic Rutaceae-feeding *Agonopterix*; posteroventral aspect, valvae reflected laterally, aedeagus removed. A, *A. paulae*. B, *A. nigrinotella*. C, *A. pteleae*. D, *A. costimacula*. Scale = 0.5 mm.

A. paulae the "plate" in the center of the anterior margin is thickened only at its lateral margins, whereas in *A. costimacula,* the entire anterior and lateral margins of the plate are thickened. Also, in *A. paulae,* the ostium bursae extends posterad almost to the posterior margin of A8, whereas in *A. costimacula* the posterior extremity of the ostium is relatively far removed from the posterior margin of the segment. Finally, the eighth abdominal sternum in *A. paulae* bears setae only in the posterolateral areas, whereas in *A. costimacula,* setae are distributed over much of the sternum.

Etymology.—This species is named in honor of Paula D. Harrison, wife of the senior author, in recognition of the immense contribution that she has made to his life and career, including valuable field assistance on some of the earliest excursions during which this species was collected.

Type series.—Holotype male: Collected as larva on *Zanthoxylum americanum,* USA: Illinois, Piatt County, University of Illinois-Robert Allerton Park, Lost Garden Trail, 28-VII-1994; iss. 28-VIII-1994 (USNM). Allotype female: same locality and dates as for holotype (USNM). Paratypes: ten specimens, with same collection data as for holotype, emergence dates from 26-VIII-1994 to 4-IX-1994 (USNM, INHS, JRW, SCP); one specimen, collected as larva on *Zanthoxylum americanum,* USA: Illinois, Menard County, 5 mi. NW of Greenview, R. & C. Baugher property, T19N, R7W, Sec. 12, 13-VII-2001, T. Harrison/J. Wiker, iss. 5-VIII-2001 (JRW); five specimens, same data as for preceding except collected by J. Wiker, 29-VII-2002, iss. 26-VIII-2002 (JRW); one specimen, collected as larva on *Zanthoxylum americanum,* USA: Illinois, Coles County, Charleston, Lake View Park, T12N, R9E, NW ¼ Sec.

24, 13-VII-2000, T. Harrison, iss. 3-VIII-2000 (INHS).

Biologies of Rutaceae-Feeding *Agonopterix* Species in Illinois

Larval food plant specificities.—Hodges (1974) reported that male genitalia of a single type were seen in specimens of "*A. nigrinotella*" reared from both *Z. americanum* and *P. trifoliata,* implying that at least one of the entities identified as *A. nigrinotella* is capable of utilizing both plants. In the present study, field-collected larvae of all four Rutaceae-feeding *Agonopterix* species displayed strict monophagy; furthermore, in no-choice food plant-switching tests done with neonates of three of the species (and with fed first-stadium larvae of the fourth species, *A. nigrinotella*), each species accepted only the food plant that it was observed to utilize in the field.

We conclude that each of the four Nearctic Rutaceae-feeding *Agonopterix* species is strictly monophagous, with *A. paulae* and *A. nigrinotella* feeding as larvae only on *Z. americanum,* and *A. pteleae* and *A. costimacula* utilizing only *P. trifoliata.* It is noteworthy that this is the same conclusion drawn by Clarke (1941), except that he apparently did not examine any specimens of *A. paulae,* as he did not recognize it as a separate species.

Hodges (1974) reported that *A. nigrinotella* has been reared from *Carya.* In the USNM collection are specimens of *A. nigrinotella* (identity confirmed by our own examination) that are labeled as having been reared in Ohio, by the late Annette Braun, from an unidentified species of *Carya.* Excepting this record, *Carya* has never been listed as food plant for *A. nigrinotella* nor for any other Nearctic *Agonopterix* species. During the present study, we attempted to rear *A. nigrinotella* on leaves of two common *Carya* species of the eastern USA, *C. ovata* (Miller) Britton and *C. glabra* (Miller) Britton. Larvae refused both species, and they fed and developed normally when transferred to *Z. americanum.* This test is not definitive, because only two *Carya* species were provided, and because fed first-stadium larvae (the earliest stage that we could obtain), rather than neonates, were used, and they might have been irreversibly conditioned to feed only on *Z. americanum* (Zalucki et al. 2002). Given, however, the uniqueness of the *Carya* record, the strict monophagy seen elsewhere in Nearctic Rutaceae-feeding *Agonopterix* (in laboratory tests involving unfed first-stadium larvae of three of the four species, and in all field observations), and the disjunct taxonomic relationship of Juglandaceae and Rutaceae, we conclude that it is doubtful that *A. nigrinotella* utilizes *Carya* as a food plant. The record of *A. nigrinotella* on *Carya* is tentatively assessed as a misidentification of the food plant, awaiting a definitive test in which neonates of *A. nigrinotella* are offered all *Carya* species that occur in Ohio.

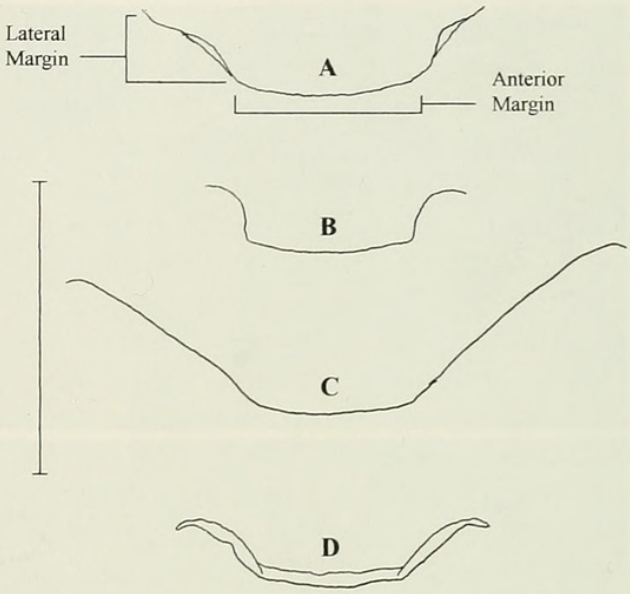


Fig. 4. Medial plates of anterior margins of eighth abdominal sterna in female adults of Nearctic Rutaceae-feeding *Agonopterix* (plate absent in *A. pteleae*); ventral aspect. A, *A. paulae.* B, *A. nigrinotella.* C, *A. pteleae.* D, *A. costimacula.* Scale = 0.5 mm.

Clarke (1941) named only "*Salix* spp." as food plant for *A. argillacea,* but Hodges (1974) also listed *P. trifoliata.* In feeding trials conducted in the present study, unfed neonates of *A. argillacea* refused *P. trifoliata*; they fed and developed normally when

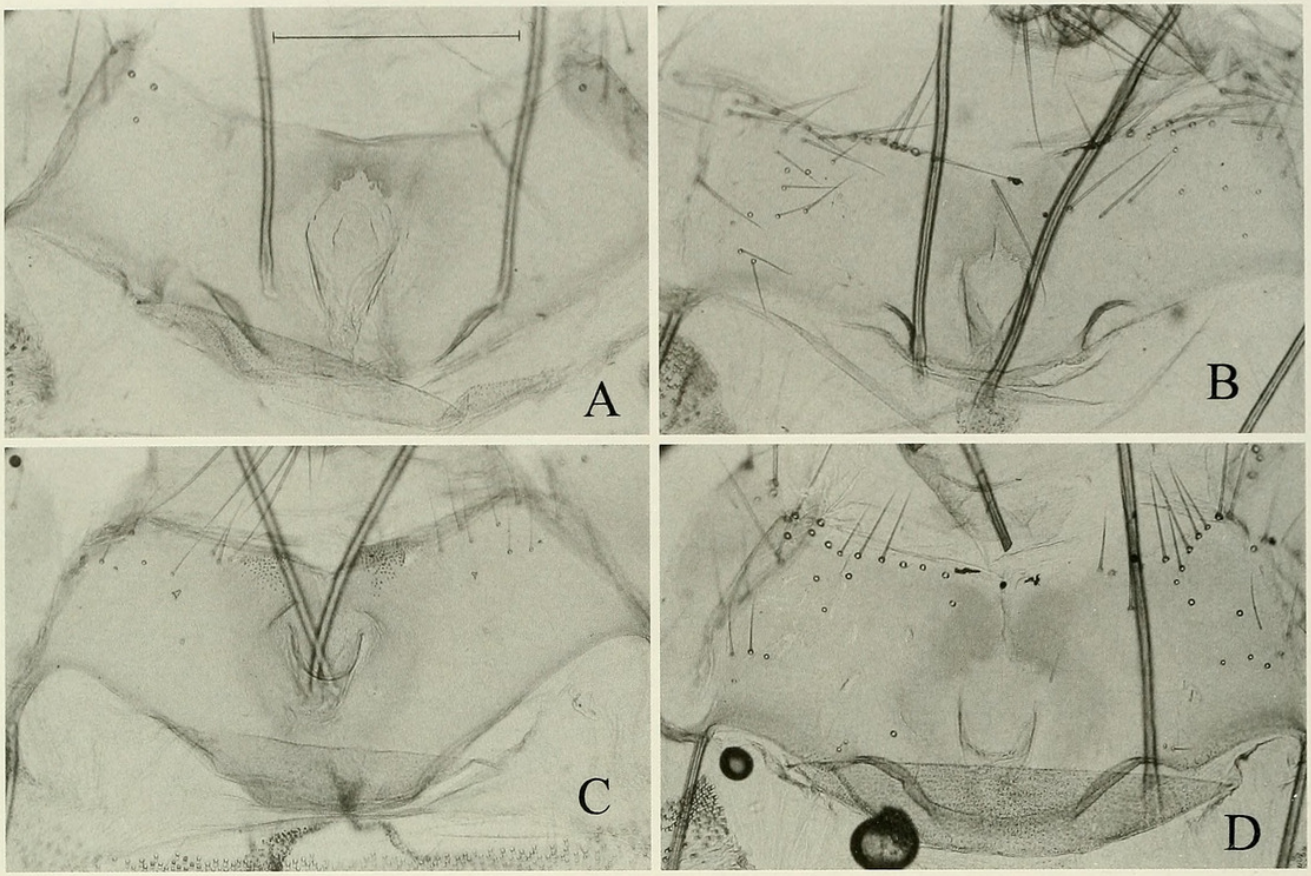


Fig. 5. Eighth abdominal sterna of female adults of Nearctic Rutaceae-feeding *Agonopterix*; ventral aspect. A, *A. paulae*. B, *A. nigrinotella*. C, *A. pteleae*. D, *A. costimacula*. Scale = 0.5 mm.

transferred to the field host, an undetermined species of *Salix* Linnaeus. We conclude that the record of *A. argillacea* on *Ptelea* is an error.

Phenology and voltinism.—L'vovskiy (1975), commenting on the European Oecophoridae (traditional sense, including Depressariinae, Amphisbatinae, and Oecophorinae), noted that almost all species are univoltine. The four exceptions include *Pseudatemelia josephinae* Toll, which requires two years per generation, and three species that go through two or more generations per year: *Endrosis sarcitella* (Stephens), a synanthropic stored grain pest; and one species each of *Depressaria* Haworth and *Amphisbatis* Zeller. L'vovskiy also outlined four different patterns of univoltinism seen in European Oecophoridae (Fig. 6A). Type 1, with wintering as adults, is seen in *Agonopterix, Depressaria,* and *Martyrhilda* Clarke. Type 2, in which partly grown larvae are the wintering stage, is characteristic of Oecophorinae, Pleurotinae, and Deuterogoniinae. Type 3, in which pupae over-winter, is seen in *Cheimophila* Hübner, *Semioscopis* Hübner, *Epigraphia* Stephens, and most *Diurnea* Haworth. Only *Diurnea phryganella* (Hübner) exhibits Type 4, in which winter is passed in the egg stage. Hodges (1974) reported that North American species of *Agonopterix* for which life histories are known are generally univoltine and follow the Type 1 program, but that a few species lay eggs in late summer, and in these the egg is the over-wintering stage (apparently similar to Type 4).

Our findings with Rutaceae-feeding *Agonopterix* in Illinois indicate that, although all four species over-winter as adults, Type 1 univoltinism is seen in only one of the four species, *A. nigrinotella* (Fig. 6B). In this species, mature larvae are present in central Illinois only from late May into very early June.

Although the time required to complete

a single generation (Fig. 6C) is similar in all four Rutaceae-feeding species, the seasonal cycles in the three species other than *A. nigrinotella* differ from Type I univoltinism. In *A. paulae,* young larvae were collected at Allerton Park, Piatt County on 3 June, 1999, at the time when *A. nigrinotella* was completing larval development. These *A. paulae* larvae matured, pupated, and emerged as adults in early July 1999. These adults did not mate in the laboratory; however, mature larvae appeared once again on *Z. americanum* at Allerton Park during late July 1999. No *Agonopterix* larvae have been found on *Z. americanum* later in the year than the late-July generation of *A. paulae.* Given the timing of these observations, it appears likely that *A. paulae* is bivoltine.

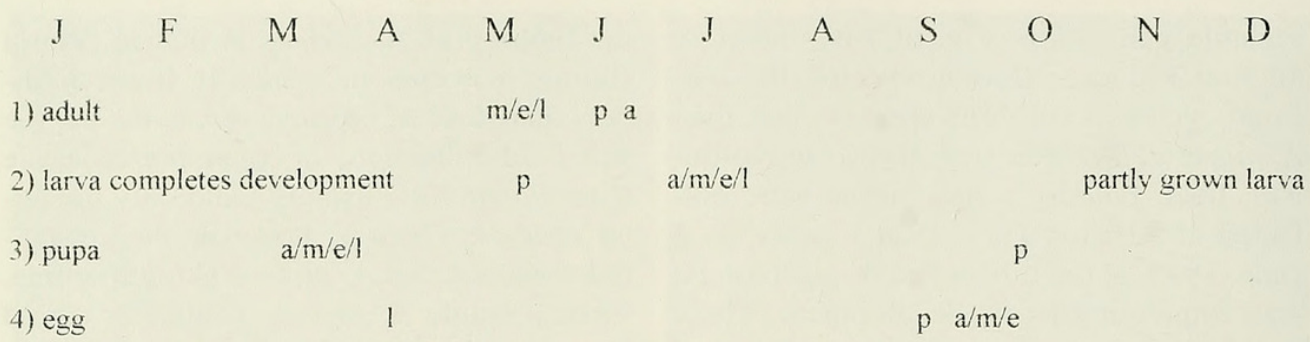
The two *Ptelea*-feeding species display a pattern that is even less readily interpreted as strict univoltinism than that seen in *A. paulae.* In central Illinois, mature larvae of *A. costimacula* appear in a fairly synchronized initial cohort in late May. Shortly afterward, larvae of all different stages can be found, and this situation remains continuously throughout the remainder of spring and summer, ending only with leaf senescence (which usually occurs in early September).

Although this pattern strongly suggests multivoltinism for *A. costimacula,* we could not summarily rule out the possibility that the different temporal cohorts of larvae that appear throughout the late spring and summer are the result of remarkably staggered oviposition by a single generation of overwintered adults. This scenario, however, was cast into doubt (at least as the sole explanation for the phenology of this species) by our observation in the laboratory of mating in a pair of adult *A. costimacula* that we had reared from early-season larvae, behavior highly suggestive of a second generation.

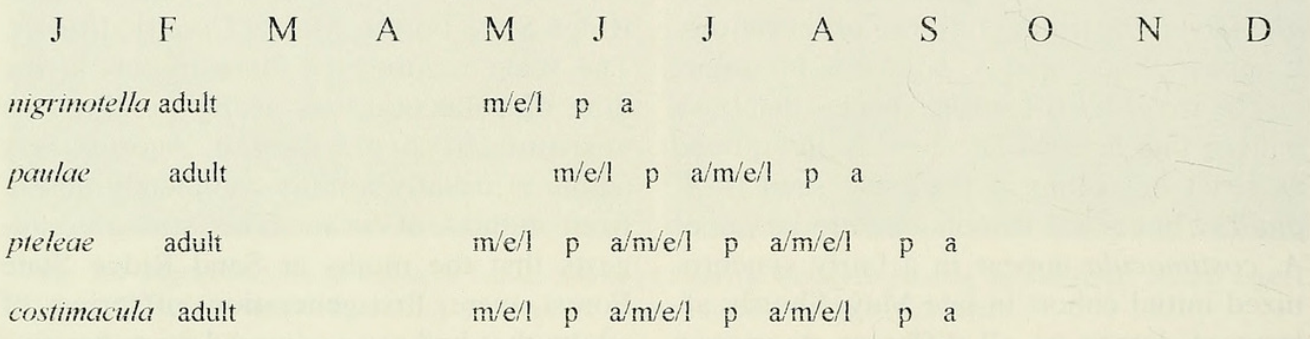
The situation in *A. pteleae* is probably the same as in *A. costimacula,* although the evidence of multivoltinism in this species is not as strong. A major difficulty in studying the biology of *A. pteleae* is that in central Illinois it occurs in markedly lower numbers than does *A. costimacula,* to the degree that field collections of *Agonopterix* larvae from *P. trifoliata* usually yield only the latter species. There is, however, circumstantial evidence that *A. pteleae* is multivoltine. First, a single *A. pteleae* adult was reared from larvae collected in late May at Forest Glen Nature Preserve, Vermilion County, Illinois; this corresponds to the initial cohort of *A. costimacula,* several individuals of which were reared from the same collection that produced the individual of *A. pteleae.* Second, adult female *A. pteleae* were collected at UV light in mid-June at Sand Ridge State Forest, Mason County, Illinois. The scale vestiture of these insects at the time of collection was nearly pristine (the vestiture of overwintered *Agonopterix* adults is usually almost completely absent from months of wear). This strongly suggests that the moths at Sand Ridge State Forest were first-generation offspring of adults that had over-wintered from the previous year, as does the timing (they were collected at the same time that moths would have appeared from the known late-May larval cohort at Forest Glen). Furthermore, when the Sand Ridge moths were caged in the laboratory, they laid fertile eggs, which the univoltine offspring of wintered adults would not have done. Third, *A. pteleae* adults emerged in late August from two pupae collected from sleeved *P. trifoliata* at Charleston, Coles County, Illinois. It therefore appears that, as in *A. costimacula, A. pteleae* is multivoltine with several generations produced from late spring through summer, although this supposition should be confirmed by observation of *A. pteleae* at a single site.

Genetic Differences in the Four Nearctic Rutaceae-Feeding Species of *Agonopterix*

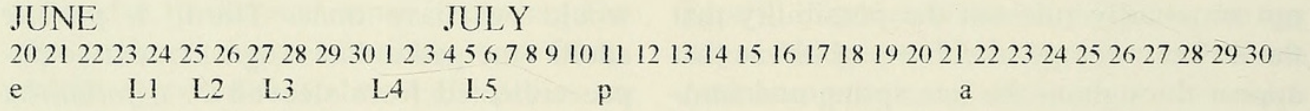
Sequences of the mitochondrial *COII* fragment from *Agonopterix costimacula, A. nigrinotella, Agonopterix pulvipennella*



A



B



C

Fig. 6. A, Four different seasonal cycles in univoltine "Oecophoridae" of Europe, as outlined by L'vovskiy (1975). B, Seasonal cycles of Rutaceae-feeding *Agonopterix* from Illinois. C, Life cycle of *Agonopterix pteleae* reared from egg to adult (four individuals, intervals averaged); similar intervals were noted in the other three species; e, egg; 1, larva, L1–L5, instars 1 through 5; p, pupa; a, adult; m, mating.

(Clemens), *A. pteleae, Depressaria pastinacella* (Duponchel), and "*Agonopterix* n. sp. #1" (= *A. paulae*) have been deposited in Genbank under accession numbers AY527038, AY527039, AY527040, AY527041, AY527042, and AY527043, respectively. PCR product from one species (*A. pteleae*) yielded only 418 bp of sequence readable in both directions, and comparisons of all four Rutaceae-feeding

Table 1. Pairwise comparisons of a 418-bp region of mitochondrial gene *Cytochrome Oxidase II* in Nearctic Rutaceae-feeding *Agonopterix*. For each species pair, numbers indicate total number of bases differing, followed by percent sequence difference (in parentheses).

	nigrinotella	*costimacula*	*pteleae*
paulae	23 (5.50)	15 (3.59)	28 (6.70)
pteleae	26 (6.22)	22 (5.26)	
costimacula	15 (3.59)		

species are therefore based on this subset (positions 1691 through 2108) of the 522-bp total. Numbers of base differences and percent sequence difference for each species pair are presented in Table 1. The 3.59–6.70% differences seen here are much larger than the 0.00–0.96% differences seen in this same region of *COII* in three species of *Yponomeuta* Latreille (Sperling et al. 1995). Our molecular results therefore align with the morphological and biological evidence in supporting the conclusion that Nearctic Rutaceae-feeding *Agonopterix* comprise four valid species.

Generally, one individual of each species was sequenced; however, a partial sequence obtained from a second individual (representing a second population) of *A. costimacula* shared 100% identity with the corresponding area of the complete, 522-bp sequence from that species. Because *COII* appears to display considerable polymorphism among species of *Agonopterix* (but apparently a high degree of intraspecific conservation, according to our limited observation), sequence data from this gene might be particularly useful in a phylogenetic analysis of the genus.

Conclusions

Four Nearctic species of *Agonopterix* utilize Rutaceae as larval food plants in Illinois. These species are clearly separable by diagnostic differences in ecological and morphological characters, and they display substantial interspecific divergence in sequence of the mitochondrial gene, *Cytochrome Oxidase II*. Each of the four Nearctic Rutaceae-feeding species of *Agonopterix* in Illinois feeds on only one of the two locally occurring Rutaceae species, as seen in feeding tests with neonates and first instars (feeding tests conducted during this study also contradict previous records of *A. nigrinotella* on *Carya* species, and of *A. argillacea* on *P. trifoliata*). Only one of the Rutaceae-feeding species, *A. nigrinotella*, displays Type 1 univoltinism (L'vovskiy 1975) as seen in most *Agonopterix*, whereas *A. paulae* is bivoltine, and both *A. pteleae* and *A. costimacula* are multivoltine.

That substantially greater diversity than was previously recognized has been found to exist in associations between *Agonopterix* and Rutaceae in central Illinois suggests that much remains to be learned about even well-studied microlepidoptera. The depressariine Elachistidae may well serve as a useful group for study of such evolutionary phenomena as host-associated speciation (Howard and Berlocher 2001).

Key to Mature Larvae of Nearctic Rutaceae-Feeding *Agonopterix*

1. Body either with dark lateral markings on segments T1 and A10 (but with no dark pinacula) (Fig. 2A), or with dark pinacula at base of D setae on most segments (but with no dark markings elsewhere on thorax or abdomen) (Fig. 2B); food plant, *Zanthoxylum americanum* 2
– Body uniformly green, with no dark pinacula or markings (Figs. 2C, D); food plant, *Ptelea trifoliata* 3
2. Body with dark lateral markings on segments T1 and A10 (Fig. 2A) but no dark pinacula; bivoltine, mature larva (central Illinois) occurring in late June, again in late July . . . *A. paulae*
– Body with dark pinacula at base of D setae on most segments (Fig. 2B), but otherwise with no dark markings on thorax or abdomen; univoltine, mature larva (central Illinois) occurring in late May/early June . . . . . *A. nigrinotella*
3. Spiracular peritremes brown . . . . . *A. pteleae*
– Spiracular peritremes black . . . . . *A. costimacula*

Key to Adults of Nearctic Rutaceae-Feeding *Agonopterix* (External Characters)

1. Dorsal surfaces of thorax and mesowing covered with upturned scales, giving these struc-

tures a "shaggy" appearance (Fig. 1C); a large, blackish blotch slightly beyond mid-length on anterior part of mesowing . . . . . *A. pteleae*

– Dorsal surfaces of thorax and mesowing smoothly scaled (Figs. 1A, B, D) 2

2. Mesowing relatively uniform brown, without prominent blackish spots along costa, and without a distinct blackish ring around the whitish discal spot (Fig. 1B) . . . . *A. nigrinotella*

– Mesowing with prominent blackish spots along costa, and with a distinct blackish ring around the whitish discal spot (Figs. 1A, D) 3

3. Mesowing ochreous brown, with a diffuse dark-gray blotch anteriorly, beyond mid length, and with blackish streaks in apical third, these coinciding with wing veins (Fig. 1A) . . . *A. paulae*

– Mesowing grayish brown, without a dark-gray blotch or vein streaks (Fig. 1D) . . *A. costimacula*

Key to Adults of Nearctic Rutaceae-Feeding *Agonopterix* (Genital Morphology)

1. Males 2

– Female 5

2. Clasper extending to or slightly exceeding dorsal margin of valve; ventrobasal fold of valve wide, exceeding 0.4× width of valve at base; gnathos subglobose, length-to-width ratio 1.5 or less (Fig. 3C) . . . . . *A. pteleae*

– Clasper not extending to dorsal margin of valve; ventrobasal fold of valve narrow, not exceeding 0.35× width of valve at base; gnathos elongate, length-to-width ratio 2.5 or greater (Figs. 3A, B, D) 2

3. Valve relatively long and narrow, length at least 2.4× width at base; clasper straight or nearly so (Fig. 3A) . . . . . *A. paulae*

– Valve relatively short and wide, length 2.1× width at base, or less; clasper strongly curved (Figs. 3B, D) 3

4. Valve narrow (length greater than 2.0× width at base), distal half of valve tapered to a narrow, acuminate apex (Fig. 3B) . . . *A. nigrinotella*

– Valve broad (length approximately 1.9× width at base), distal half not tapered to a narrowly acuminate apex (Fig. 3D) . . . . . *A. costimacula*

5. Eighth abdominal sternum (hereafter referred to as A8) with anterior margin forming an even curve, without a medial plate that is differentiated from the rest of the margin (Figs. 4C, 5C) . . . . . *A. pteleae*

– A8 with anterior margin bearing a medial plate that is differentiated from the rest of the margin (Figs. 4A, B, D, 5A, B, D) 2

6. Lateral margins of medial plate of A8 strongly and evenly curved; medial plate relatively narrow, occupying approximately 45 per cent of total width of A8 at anterior margin; anterior margin of medial plate relatively wide, occupying about 65 per cent of total width of plate (Figs. 4B, 5B) . . . . . *A. nigrinotella*

– Lateral margins of medial plate of A8 straight or nearly so, not strongly curved; medial plate relatively wide, occupying approximately 55 per cent of total width of A8 at anterior margin; anterior margin of medial plate relatively narrow, occupying about 45 per cent of total width of plate (Figs. 4A, D, 5A, D) 7

7. Lateral and anterior margins of medial plate uniformly thickened; ostium relatively small, extending posterad, midventrally, to less than 70 per cent of the distance from anterior to posterior margins of A8; A8 with at least 20 setae, which are not restricted to posterolateral area of sternum (Figs. 4D, 5D) . . *A. costimacula*

– Lateral and anterior margins of medial plate not thickened, except in a small area of each lateral margin; ostium relatively large, extending posterad, midventrally, to more than 85 per cent of the distance from anterior to posterior margins of A8; A8 with fewer than ten setae, all restricted to the posterolateral areas of the sternum (Figs. 4A, 5A) . . . . . *A. paulae*

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