THE SIGNIFICANCE OF THE EGG STAGE TO TAXONOMIC AND PHYLOGENETIC STUDIES OF THE EPHEMEROPTERA

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Through a study of the eggs of approximately 100 of the known mayfly genera, it is evident that the egg stage in this group of insects can provide valuable data for taxonomic and phylogenetic studies of the order.

Almost unique to the mayflies is the fact that most adult females lay their eggs freely on the surface of the water rather than personally attaching them to any fixed objects. To insure survival and aid dispersal, the egg itself must have some means of attachment to submerged objects. For this purpose many elaborate chorionic structures have evolved which enable mayfly eggs to adhere to submerged surfaces. These chorionic structures are appropriately called "attachment structures." and they presumably prevent the eggs from being washed into an unfavorable part of the aquatic environment.

Dispersal of the eggs is most likely assisted when some of them adhere to submerged surfaces shortly after deposition, while others float further downstream before attachment. Survival of the eggs is most likely aided by their attachment in a place in contact with flowing water, rather than their settlement in a backwash, eddy or some other area continuously subjected to siltation. Maximum survival of the species is of course insured by the dispersal and survival of as many eggs as possible.

It is important to realize that many lake species are also equipped with attachment structures, and here lake currents must serve to disperse eggs from the oviposition site and into contact with submerged objects to which they adhere.

The micropyle is the structure which allows sperm to enter the egg, and it too is useful in systematic studies of the Ephemeroptera. On mayfly eggs, the evolution of the many different attachment structures has caused the micropyles to become elaborately modified in order to permit the sperm to find a micropylar opening amidst the various chorionic appendages and/or adhesive layers. The modifications basically result in a micropyle with two parts : an inner micropylar canal, and an outer sperm guide. The junction of these two parts is called the micropylar opening. The micropyles can be grouped into three types, depending upon their overall shape or appearance, and variations occur within each type.

In addition to attachment structures and micropyles, chorionic sculpturing is a feature which usually varies at one taxonomic level or another. The small ridges, tubercles and other ornamentation found on the surface of the chorion are known as chorionic sculpturing, and they can be quite useful for taxonomic purposes, or in generic and specific level phylogenetic studies. However, it appears that chorionic sculpturing arose independently in nearly every family and subfamily, and therefore it is useless as a tool for reconstructing ephemeropteran phylogeny at the family level.

One very important and outstanding feature of the mayfly reproductive cycle makes the egg stage ideally suited for systematic studies. Unlike most insect groups, mayfly eggs mature

during the nymphal or immature part of the life cycle, and therefore, eggs can be dissected from mature, black wing-padded nymphs for study. Also, those eggs may be compared to eggs dissected from adult specimens, and this procedure can be very helpful when trying to associate nymphs with adults when rearing is not possible and one of these stages is undescribed. However, one be cautious when using this procedure.

Because of the egg laying behavior of the adult mayfly, and the environment into which the eggs are released, it seems that these eggs are quite strongly subjected to the pressures of natural selection. These selection pressures have caused the evolution of a great variety of external features on Ephemeroptera eggs, and forces me to believe that studies of the egg stage can contribute valuable information to phylogenetic research on the order. I attempted in this study to show the phylogenetic relationships evident in the egg stage alone, relying on adult and nymphal evidence to the barest minimum. The egg stage is perhaps not as valuable as nymphal and adult stages for two reasons. The eggs have relatively fewer morphological characters, and also the eggs are rarely as long lived as the nymphs and never as active as the nymphs and adults. Nevertheless, morphological features found on the eggs are of definite systematic importance because survival of the egg is as important to survival of the species as is survival of the nymphs and adults.

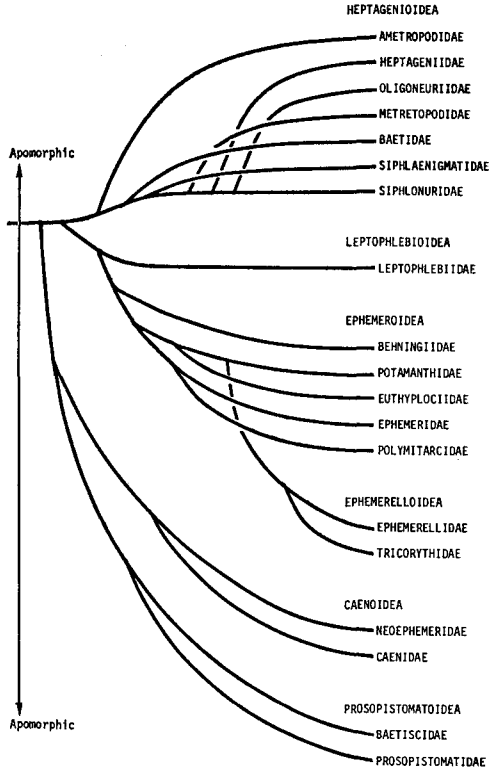

FIGURE 1. Diagrammatic representation of the probable phylogenetic relationships of the families of Ephemeroptera, Palingeniidae not included.

The phylogenetic diagram (Fig. 1) portrays my interpretation of the data collected in this study of mayfly eggs. Total egg data suggests a classification which is similar to those published by EDMUNDS and various coworkers since 1954. The classification proposed herein, however, makes a few changes over these earlier classifications. The Ephemerellidae and Tricorythidae are placed in a superfamily (Ephemerelloidea) distinct from the Leptophlebioidea, and are considered as possibly having been derived from the Potamanthidae. The polar caps, other attachment structures and micropyles are too similar in the Potamanthidae and Ephemerelloidea to allow easy acceptance of the hypothesis of independent origin of this combination of characters, and thus independent origin of the two lineages. The Metretopodinae of those earlier classifications is raised to the family level, and the Baetidae are considered to have had an earlier origin than that suggested by EDMUNDS (1962).

Within the evolution of the Siphlonuridae is found the derivation of six other families of the Ephemeroptera : Ametropodidae, Baetidae, Siphlaenigmatidae, Metretopodidae, Heptageniidae and Oligoneuriidae. Together with the Siphlonuridae, these families comprise the superfamily Heptagenioidea.. Data collected in the study of Heptagenioidea eggs show quite vividly many of the steps probably involved in the evolution and distribution of certain types of micropyles and attachment structures.

The classification suggested by egg data is entirely unlike those proposed by DEMOULIN in various publications since 1958. Neoephemeridae eggs do not in any way indicate a relationship of that family to the Ephemeroidea; there is no basis for placing the Rallidentinae, Coloburiscinae and Isonychiinae in a separate family (Isonychiidae) and including them in a superfamily (Oligoneurioidea) distinct from the Siphlonuridae and Heptagenioidea; eggs of Baetiscidae and Prosopistomatidae show no affinities to any of those in the families and subfamilies which DEMOULIN places in his Siphlonurioidea; the Leptophlebiidae are most certainly not of close enough relationship with the Heptageniidae to include both families in the same superfamily; and eggs of Caenidae show no basis for including this family in the Ephemerelloidea.

RÉSUMÉ

Signification du stade œuf dans les études taxonomiques et phylogénétiques des Éphéméroptères

On utilise trois groupes de caractères morphologiques dans l'étude des œufs d'Éphéméroptères.

1. Structures d'attachement : elles servent à l'attachement des œufs aux objets immergés dans les fleuves et les lacs.

2. Micropyles : les structures permettant l'entrée du sperme dans l'œuf sont les micropyles, ceux-ci se composent généralement d'un canal micropylaire interne et d'une conduite de sperme externe. Leur jonction constitue l'orifice micropylaire.

3. Les structures chorioniques : les petites crêtes, tubercules et autres ornementations trouvées à la surface du chorion constituent les structures chorioniques.

Bien que les œufs aient moins de caractères morphologiques que les stades larvaires ou adultes, ils sont d'une importance capitale pour la survie de l'espèce et par conséquent fournissent des informations valables du point de vue taxonomique et phylogénétique. Parce que les œufs sont à maturité chez la larve mature, ceux-ci peuvent être utilisés dans certains cas pour associer les larves et les adultes.

Le diagramme phylogénétique (Fig. 1) est basé principalement sur la morphologie de l'œuf, tout en utilisant un minimum de caractères adultes et larvaires. La figure 1 diffère de la classification de EDMUNDS et de ses collaborateurs de la façon suivante : les Ephemerellidae et les Tricorythidae sont placés dans une superfamille Ephemerelloidea distincte des Leptophlebioidea et sont considérés comme ayant été dérivés des Potamanthidae ; les Metrepodidae sont une famille distincte des Ametropodidae ; et les Baetidae semblent avoir une origine plus ancienne que celle suggérée par EDMUNDS en 1962. On y trouve aussi de nombreux points de désaccord avec les diverses hypothèses de DEMOULIN concernant l'histoire phylogénétique des Éphéméroptères.

ZUSAMMENFASSUNG

Bedeutung des Eistadiums in taxonomischen und phylogenetischen Studien der Ephemeroptera

Drei Gruppen von morphologischen Charakteren sind in der Studie von Eiern der Ephemeroptera gebraucht :

1. Anhaftungsorgane, diese dienen dazu die Eier an überschwemmte Objekte in Flüssen und Seen zu befestigen.

2. Micropyle. Die Organe, welche den Spermien erlauben in das Ei einzudringen, sind die Micropyle, und sie sind gewöhnlich aus einem inneren Micropylenkanal und einer äusseren Spermienleitung zusammengesetzt. Die Verbindung dieser 2 Teile ist die Micropyleöffnung.

3. Chorionische Skulptur. Die schmalen Furchen, Knötchen und andere Verziehrungen, welche an der Oberfläche des Chorions gefunden werden, sind als chorionische Skulptur bekannt.

Obwohl Eier weniger morphologische Charaktere als Nymphen oder Erwachsenenstadien haben, sind sie ausgesprochen wichtig für das Weiterleben der Arten, und geben darum wertvolle taxonomische und phylogenetische Auskünfte. Weil Eier in vollentwickelten Nymphen reifen, können in einigen Fällen die Eier gebraucht werden, um Nymphen und Erwachsene zu assoziieren.

Das phylogenetische Diagramm (Fig. 1) ist hauptsächlich auf Eimorphologie gegründet, wobei ein Minimum von Erwachsenen- und Nymphendata gebraucht wurden. Figure 1 unterscheidet sich von der Klassifikation von EDMUNDS und Mitarbeitern hauptsächlich in diesen Hinsichten : Die Ephemerellidae und Tricorythidae sind in die Superfamilie Ephemerelloidea plaziert worden, verschieden von den Leptophlebioidea, und stammen möglicherweise von Potamanthidae ab ; die Metretopodidae ist eine Familie verschieden von Ametropodidae, und die Baetidae scheint einen früheren Ursprung als den von EDMUNDS in 1962 vorgeschlagenen zu haben. Es bestehen auch viele Widersprüche mit den verschiedenen Hypothesen von DEMOULIN über die phylogenetische Geschichte der Ephemeroptera.

DISCUSSION

R. ALLEN : I tend to agree with you on the separation of Ephemerellidae and Tricorythidae into a distinct subfamily. I would like Dr. EDMUNDS or Dr. PETERS comments on this separation.

G. EDMUNDS : My personal feeling about the evolutionary relationships is that the ancestral form gave rise to the cluster of families Tricorythidae and Ephemere'lidae, which are very close together, and the close ally, Leptophlebiidae. But I believe the Leptophlebiidae are almost

prototypes, or perhaps ancestral types, of the whole superfamily Ephemeroidea, and this points out the difficulty in trying to translate phylogenetic relationships into classification. It is which ever way you decide to put it. It is easier to understand how things are related to one another than it is to decide on what classification system is most convenient for grouping them. I wouldn't be unhappy to remove the Leptophlebiidae and place them in a separate superfamily next to the Ephemeroidea.

P. Tsui : For a given species is the egg structure of the mature nymph and the female imagos similar ?

R. Koss : Yes, it is. The eggs are mature in the late instar nymphs and they have the same characters found on eggs dissected from adult females.

P. Tsui : Can they be used for associating nymphs and adults ?

R. Koss : Yes, if done cautiously. In most families, eggs are usually distinct enough at the generic level to make such associations, especially in small genera. In large genera such as *Rhithrogena* and *Heptagenia*, associations usually cannot be made, but they are not necessary since the generic features of the nymphs and adults of most large genera are well known. At the species level, eggs are frequently not so distinctive as to risk such an association, especially without other supportive data. This also is mostly related to the size of the genus — for example, not all *Paraleptophlebia* eggs are distinguishable at the species level, but *Hexagenia* eggs are distinct for most species.

W. Peters : Is your phylogeny, as you have shown, based entirely upon unit characters which you have found in the eggs or are you including other data that you know from other systems — like external morphology ?

R. Koss : I made a definite attempt to restrict my conclusions to the data that I collected from the eggs. I feel that if we work in isolation and come up with a result and a probable answer, then get together and find we all have the same results, that strangthens our theories. So, for the most part, I attempted to form my results and draw my diagrams based on egg data. One cannot do this exclusively because in some cases it was rather obvious to me that a particular relationship that I thought was possible while looking at the eggs was not really possible when examining the nymphs and adults.

V. Puthz : Are there different attachment structures in different habitats ?

R. Koss : Not entirely. *Caenis*, for example, is typically a pond dweller and *Caenis* has some of the most elaborately developed polar caps of any group of mayflies. I think that lake currents function just as stream currents do. The eggs are very tiny and when the eggs are laid they do not drop to the bottom like a rock. Lake currents disperse the eggs. I think pond species more generally have adhesive layers than do stream species, but some pond species also have these other apomorphic attachment structures.

G. Edmunds : I would like to make a comment about the *Caenis* because your data on the eggs show one rather exciting thing in classification. I have grouped the Neoephemeridae and the Caenidae together. I have not only decided in fact that the Caenidae are closely related to the Neoephemeridae, but I have even decided to what group in the Neoephemeridae they are related. While the *Caenis*-type egg doesn't show up in some of the Neoephemeridae it does occur in the genus *Potamanthellus* which is a neoephemerid. You have the fully apomorphic type of egg developed in *Potamanthellus* which is an ancestral form, in a sense, to the Caenidae. *Potamanthellus* is a stream dweller so this egg evolved originally in the stream. The Caenidae, to a large extent, have moved to the pond habitat but they have not lost their apomorphic

stream dwelling structures. They probably would have never picked up such structures because there would have been no strong selection for this, had they never been in a stream situation.

R. Koss : That is a good point. I wasn't aware of that.

REFERENCES

DEMOULIN, G. (1958). Nouveau schéma de classification des Archodonates et des Éphéméroptères. *Bull. Inst. Sci. Nat. Belg.* **34**(27) : 1-19.

——, (1961). Encore les Behningiidae (Ephemeroptera). *Bull. Inst. Sci. Nat. Belg.* **37**(41) : 1-6.

——, (1969a). Sur la position systématique et phylogénétique des Rallidentinae (Ephemeroptera). *Bull. Inst. Sci. Nat. Belg.* **45**(15) : 1-5.

——, (1969b). Remarques critiques sur la position systématique des Baetiscidae et des Prosopistomatidae (Ephemeroptera). *Bull. Inst. Sci. Nat. Belg.* **45**(17) : 1-8.

EDMUNDS, G.F., Jr. (1962). The principles applied in determining the hierarchic level of the higher catagories of Ephemeroptera. *Syst. Zool.* **11** : 22-31.

EDMUNDS, G.F., Jr., R.K. ALLEN and W.L. PETERS. (1963). An annotated key to the nymphs of the families and subfamilies of mayflies (Ephemeroptera). *Univ. Utah Biol. Ser.* **13**(1), 49 pp.

EDMUNDS, G.F., Jr. and J.R. TRAVER. (1954). An outline of a reclassification of the Ephemeroptera. *Proc. Entomol. Soc. Wash.* **56** : 236-40.