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STUDIES ON THE BIOLOGY OF THE EPHEMEROPTERA. I. COLORATION AND ITS RELATION TO SEASONAL EMERGENCE.*

BY HERMAN T. SPIETH,
College of the City of New York.

The following observations and conclusions are based upon ephemerids from temperate North America and principally upon material from the eastern United States. It is perfectly conceivable that in other parts of the world, where the fauna and also the environmental conditions are different, the conclusions reached here may not be applicable.

Colors in species of this order vary from white through yellow, orange, red (a few subdued reds), brown to black. The commonest colors are the browns, especially various dark browns. A few species have pale greens, but these are evanescent. There are no bright greens, blues or reds. In other words, the bright metallic colors found in some of the Odonata, Coleoptera and the Lepidoptera are lacking. A survey of the whole order shows that there are few bright colored ephemerids. Most of them are distinctly drab and the colors are usually dull and subdued. This is especially true of the more primitive species.

It is necesary to digress briefly concerning the question of primitive and specialized genera and species. By primitive, it is not assumed or meant that such groups are primitive in all characters and, conversely, by specialized it is not to be taken that these groups are specialized as far as every character is concerned. What is meant is that the sum total of all nymphal, subimaginal and adult characters indicates that the particular genus or species is more primitive or more specialized than other genera or species that it can be compared with. Naturally the conclusion as to whether a character is specialized or primitive is determined by studying it in numerous extant species as well as from the fossil evidence. Thus a species or a genus may be primitive in most characters but specialized in others. After all, a good many million years have intervened between the modern forms and the ancient ones, and the surprising fact is that any stock should still possess primitive characters after so many countless generations.

DISTRIBUTION OF COLOR IN IMAGO

By carefully clearing away all tissue that underlies the exoskeleton, it is easily proved that in the greater number of species of ephemerids the entire exoskeleton is transparent. If color is present in the chitin, it always is some shade of olive brown. Often the exoskeleton is similar to smoked glass, i.e., transpar-

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ent with a variable amount of brown. In some instances, such as the mesothoracic notum of *Leptophlebia cupida* Say and *Paraleptophlebia* spp., the brown pigment is so intense that the chitin is almost opaque. Invariably, if color is present, it is most intense on the thoracic nota. It also is rather evenly distributed throughout the entire exoskeleton, and as a result is not responsible for sharp, clear cut color patterns. Sometimes in connection with the underlying colors, it forms the typical color pattern as in *Leptophlebia cupida* Say and *Hexagenia* spp. In *Isonychia bicolor* Wlk., however, the chitin is faintly tinged with a brown color which is completely, or almost completely, masked by the underlying red color.

The tissues immediately underlying the exoskeleton are often impregnated with pigments of some sort which are mainly responsible for the distinctive color patterns of most species. The dark color patterns of the abdomens of the members of the genus *Stenonema* as well as other characters such as the banding of the femora are due to these underlying colors. Likewise the red color of *Isonychia bicolor* Wlk. is found in the tissues that underlie the chitin.

The underlying colors, as well as the various shades of brown found in the exoskeleton, are not destroyed by drying or immersing in preserving fluids. Probably they are of protein origin.

The white colors found in ephemerids are caused by two distinct factors:

1. In all adults there is present, underneath the chitinous exoskeleton and the epidermis but external to the musculature, a chalky white substance. This is localized mainly in the last three abdominal segments, the vertex of the head, and the sutures and articulating membranes of the thorax. There is considerable individual and specific variation in the distribution of this substance, and one may expect to find it underlying almost any part of the exoskeleton except the places where the muscles themselves are attached. Since the musculature of the adult is greatly reduced, this allows for a wide distribution of the substance. Just what the nature of the substance is, I can not say. It is, however, easily shown that it does not have the same refractive index as chitin. Probably it is a byproduct of the degeneration of the digestive tract and musculature of the nymph that takes place just previous to emergence. If the substance underlies a transparent area of the exoskeleton, it gives that region an alabaster white appearance. It also forms combination colors where the overlying chitin or epidermis is pigmented. In combination with some browns it results in oranges, yellows, rusts, etc. It is important to note that this type of white is present in the dark species such as members of *Siphlonurus* and *Parameletus,* but is completely masked by the dark overlying pigments.

2. Certain species such as *Ephoron album* Will., the various species of *Campsurus* and *Potamanthus* and the abdomen of *Ephemera guttulata* Pict., have a milky appearance which is found not only on the body but also on the wings of some species. This is definitely a physical color. If these specimens are immersed in a medium having a refractive index of 1.55 to 1.6, the white areas become transparent and lose their white color. If removed from the medium, they immediately regain their color. Since the chalky white substance is present in all specimens, it is not surprising to find that the two types of white combine to

help form the typical color pattern. Thus in the males and spent females of *Ephemera guttulata* Pict., the white of the last three abdominal segments is due to chalky substance, while that of segments 2-6 is a physical white. In the unspent females, the egg mass obscures this effect to some extent.

In addition there are some oranges, bright yellows and greenish yellows present in some species. They are mainly restricted to the large dorsal compound eyes and the egg masses. Invariably they are completely or almost completely destroyed by immersion in preserving fluids. To a lesser extent they also deteriorate in dried specimens. These particular pigments are probably chlorophyll derivatives.

It should again be emphasized that great areas of the exoskeleton of many species are transparent. In numerous other cases the chitin has brown pigments present in it but is still semi-transparent. In such cases the color of the underlying tissues or deposited materials enters into the make-up of the color of a particular area. In *Potamanthus myops* Walsh, for example, there are ferruginous markings on the head, the abdomen, tips of the leg joints, and the joinings of the caudal appendages. Such markings are due to pigment in the chitin. About 85 per cent of the body is covered by transparent chitin and derives its color, if any, from the underlying materials, especially muscles as in the thorax and the physical and chalky whites mentioned above.

In other instances, as in species of the genus *Stenonema*, some of the rufous colors are due to the chalky white substance underlying semi-transparent chitin that is impregnated with a light brown pigment.

Most mayfly color patterns are consequently due to a combination of various types of colors.

COLOR PATTERN

Besides actual colors, it is also necessary to consider the color pattern. The latter appears to have developed from a segmental plan in which each segment possessed the same pattern. The fusion of the head and thoracic segments have obscured the segmental nature of the pattern in these areas, but the abdomens of many species show it quite distinctly.

What the coloration of ancient mayflies was, we do not know, but judging from primitive genera such as *Siphlonurus* and *Ameletus* they were probably colored some dark shade of brown and the general pattern of the abdomen gave an annulate appearance. The wings were probably for the most part hyaline, with dark venation. Quite possibly, the base of the wing membranes in some species was tinted brown. The legs, genitalia and caudal filaments were a brown color similar to that of the body proper. The two sexes had a similar coloration.

From such a generalized condition there have been three distinct trends in ephemerid color evolution:

1. The pigments are fairly uniformly distributed over the body of the insect so that the specimen appears an even dark hue. Such a condition is characteristic of most species of the genus *Choroterpes*, of *Leptophlebia cupida* Say and of *L. nebulosa* Wlk., as well as many species of other genera such as *Ironopsis, Rhithrogena, Isonychia* except for the legs, *Habrophleboides* and *Parameletus*.

2. The cuticular pigments are greatly reduced, sometimes almost lacking, over the entire body. In such instances the color is dependent upon the underlying tissues and often the specimens appear very light. In extreme instances, the individuals appear almost white. Such darker pigments as are present are restricted to the dorsal surfaces of the body, especially the dorsum of the thorax, the last three tergites of the abdomen, the head—particularly the compound eyes, and the longitudinal wing veins. Extreme examples of this type of modification can be found in *Campsurus, Ephoron, Pentagenia, Caenis, Oligoneuriella,* and *Potamanthus.* Less extreme cases are the pulchellum complex of *Stenonema* and the invaria complex of *Ephemerella.* There are all degrees of intergradation between the primitive colorattion and the highly specialized types as exhibited by *Campsurus.*

3. A large number of species display what can be called the baetid type of coloration, where there is a clear-cut sexual dimorphism in regard to color. In the males the pigmentation is completely lacking from some areas, while other parts are heavily pigmented. It should be remembered that just previous to emergence time and also to some extent during the subimaginal period, extensive changes take place in the digestive system of the ephemerids. The changes result in the mesenteron being converted into a thin-walled, transparent air bladder. Such changes are not restricted to the digestive tract and, in many instances, all the muscles of abdominal segments 2-7 entirely disappear. There is considerable individual variation in such degeneration of the abdominal muscles, so that some individuals possess underlying muscle tissue in these abdominal segments while other members of the same species, collected from the same nuptial flight, completely lack it.

In the males of many species of *Paraleptophlebia, Baetis, Pseudocloeon, Cloeon,* the head, thorax and the last three or four abdominal segments are deeply pigmented, while the wings, abdominal segments 2-7, the caudal filaments and the legs are hyaline. In other species, such as *Cinygmula atlantica* McD., *Paraleptophlebia bicornuta* McD., *P. heteronea* McD., and *Habrophlebia vibrans* Need., the pigments are deposited in a definite pattern in the abdominal segments so that the abdomen appears only semi-transparent.

The wings, legs, and caudal appendages of the females are pigmented similarly to those of the males, but the entire body is uniformly pigmented and lacks all transparent areas.

COLORATION OF THE SUBIMAGO

Any consideration of ephemerid coloration would be incomplete without mentioning the subimago. This invariably presents a dull, soft opaque appearance. In the air the living individuals are invariably entirely opaque. The difference in texture between a subimago and imago is similar to the difference between a matte and a glossy finish in photography.

The pellicle that is shed by the subimago varies from white in some species to a blackish grey in others. If one of the white but opaque casts, as in *Potamanthus myops* Walsh, is placed in a medium that has the same refractive index as chitin, it immediately becomes transparent. The same thing happens with the grey pellicle of other species such as *Leptophlebia cupida,* Say except that a rather uniformly distributed melanistic pigment is clearly evident. Thus the actual opacity of the subimago is due to physical effects, but there are often pigments involved which result in combination coloration.

There are a few species, for example *Baetisca obesa* Say, where the subimago has a color pattern somewhat different from that of the imago due to the deposition of dark patches of pigmentation in the subimaginal cast. Usually this is not the case and any pigmentation that is deposited in the subimaginal pellicle is uniformly distributed.

EMERGFNCE TIME

In the region of New York City, the earliest species to emerge is *Leptophlebia cupida* Say, whose adults appear in early April. The latest species, or in some instances the second generation of some of the early emerging species, have adults appearing in late October and early November. *Paraleptophlebia debilis* Wlk. is an example of a late season emerging type. Most of November, all of December, January, February, and March are without the emergence of a single species.

The peak of emergence, so far as number of species is concerned, arrives in late May and early June. By the end of June, the number of species emerging is very small and continues so throughout the remainder of the emergence season. In northern latitudes, the emergence period is shorter and the peak of emergence is later. At Churchill, Manitoba, *Leptophlebia nebulosa* Wlk. emerges about the first of July. This species is the northern relative of *L. cupida* Say and in the southern part of its range is found in the same area as *L. cupida* Say and emerging at the same time. It is well to remember that it is the rate of development of the nymph that determines when the imago shall emerge. Development seems to be entirely dependent on the water temperatures.

The relation of rate of development to temperature explains the fact that in the western part of North America, where large streams are fed by huge melting snow fields high in the mountains, that the swiftly flowing streams are cool even after they reach the hot plains, and as a result the peak of emergence is late in the summer, i.e., late July and August.

In the southern part of the United States, the length of the emergence period of the group is much longer. There seems to be no reason why in the tropics and subtropics there should not be some species emerging during each month of the year. As far as I can determine from published records, this is true.

Regardless of the time of emergence during the year, each species has a definite limited period of emergence. In those species which have more than one generation each year, naturally there is more than one emergence period. Usually the period of emergence is relatively short. There are some species, however, such as *Stenonema tripunctatum* Banks and *Isonychia bicolor* Wlk., which emerge during the entire summer. These are the exceptions and not the rule. Ide, in his splendid paper dealing with temperature and the distribution of the mayfly fauna in a stream system, has definitely shown that in the upper part of a stream where the water temperatures were lower and more uniform that the period of emergence for a particular species is much longer than it is for the same species further downstream where the water temperatures fluctuate much more and are much higher during spring, summer, and fall. Thus there is considerable variation as to the length of emergence time of a single species at different levels within a single stream system. For a fuller discussion of the subject of temperature, the reader is referred to Ide's paper (Univ. of Toronto Studies, Biological Series, No. 39: 3-77).

SEASON OF EMERGENCE AND COLOR

In the eastern part of the United States on the latitude of New York City, the first species to emerge in the spring (late April and early May) are *Leptophlebia cupida* Say, *Paraleptophlebia adoptiva* McD., and *Baetis frivolus* McD. In the southern part of its range, *Leptophlebia nebulosa* Wlk. also emerges at the same time. All of these species emerge before the deciduous trees have leafed out. All have dark venation, dark bodies and appendages. Before they have finished their emergence periods and before the leaves of the deciduous trees have reached full size (middle-two-thirds of May). *Ameletus ludens* Clem., *Iron pleuralis* Bks., *Siphloplecton basale* Clem., *Collibaetis americanus* Bks., and *Ephemerella subvaria* Wlk. appear. These latter species are also all darkly colored. At the end of this period, the first representatives of the genus *Stenonema* are to be found, i.e., *S. vicarium* Wlk., *S. pudica* Hag., and, in areas where the streams flow through limestone country, *S. femoratum* Say. These likewise are all dark colored species.

During late May and all through June, a host of species of various genera appear in great numbers, led by species of *Isonychia, Iron, Stenonema, Ephemera, Ephemerella, Paraleptophlebia, Baetis, Cloeon, Pseudocloeon, Baetisca, Siphlonurus, Heptagenia,* and *Cinygmula*. Many of these are heavily pigmented with dark pigments. It is of interest, however, that the close relatives of those species which emerged earlier in the season are much lighter. Thus, *Leptophlebia johnsoni* McD. is lighter than *L. cupida* Say or *L. nebulosa* Wlk.; *Callibaetis ferrugineus* Walsh than *C. americanus* Bks. *Iron humeralis* Morg. and *I. suffusus* Trav. are lighter than *I. pleuralis* Bks.; *Ephemerella invaria* Wlk. and *E. rotunda* Morg. than *E. subvaria* McD.; while *Stenonema vicarium* Wlk., *S. pudica* Hag. and *S. femoratum* Say are darker than any other known species of the genus. *Ameletus ludens* Clem. and *Siphloplecton basale* Clem. have no close relatives. In the cases of *Paraleptophlebia adoptiva* McD. and *Baetis frivolus* McD., we find that the relatives which emerge later are not only lighter in their venation, legs and caudal appendages but, in the case of the males, abdominal segments 2-7 are light, often being transparent or semi-transparent.

A further inspection of the various genera shows that, where there is a genus that has species emerging throughout the season, the earliest species to emerge is darker than species which follow later. Thus, in the pulchellum group of *Stenonema, S. vicarium* Wlk. and *S. pudica* Hag. are darker than all later forms of which *rubromaculatum* Clem., *ruber* McD., *pulchellum* Walsh, *bipunctatum* McD., and *mediopunctatum* McD. are examples. In the tripunctata complex of the same genus, *femoratum* Say is darker than *tripunctata* Bks., while again in the same genus in the interpunctata complex, *canadense* Wlk. and *gildersleevei* Trav. are darker than any other species in the group. In the invaria complex of *Ephemerella, E. subvaria* McD. is darker than any other species of the same complex. *Subvaria* McD. is followed by *invaria* Wlk. and *rotunda* Morgan which are in turn much darker than *dorothea* Need. which follows still later. Turning to *Paraleptophlebia,* we find that the early eastern *adoptiva* McD. is darker than any other eastern species that emerges later. Finally, still later in the season, July and August, there emerge those forms which are almost completely light, such as *Potamanthus, Caenis, Ephoron,* and, in the middle of the United States, *Campsurus.*

There are, of course, exceptions to this generalization. The exceptions can be divided into three categories: (1) the primitive genera such as *Siphlonurus,* all species of which emerge during a rather short period and are dark; (2) certain genera such as *Hexagenia, Chorotcrpes, Habrophlebia, Habrophleboides, Cinygmula, Rhithrogena, Ironodes, Iron pis,* and *Tricorythodes,* the species of which are all dark with few exceptions; (3) sections of large genera such as *Ephemerella* and *Ephemera.* Thus the fuscata and bicolor groups or complexes of *Ephemerella* are exceptions but the *E. invaria* group as shown above does fit into the generalization. It should be noted that the *E. invaria* group seems to be the most primitive in the genus. In *Ephemera* both *E. blanda* Trav. and *E. varia* Etn. which emerge later than *E. simulans* Wlk. are pale colored species but *E. guttulata* Pict. emerges as early as or earlier than *E. simulans* Wlk. and is decidedly white except for the thorax, head, and certain other areas.

Before attempting to determine the reasons involved in this distribution of color patterns throughout the group, it is necessary to consider the daily emergence and mating time of the various species.

Those species which emerge in the early part of the season invariably do so during the daytime, and likewise the nuptial dance occurs during the day. As the season progresses, there is a tendency to push the emergence time toward late evening so that, from the early part of June, many species tend to emerge at night.

In the case of the nuptial dance, there are two tendencies:

1. Most of the species and especially those that have large individuals, such as the members of *Stenonema, Heptagenia,* and *Hexagenia,* and those that are completely light (*Potamanthus, Caenis*) tend to have the nuptial dance at dusk or even still later at night. The apex of such development is reached in such genera as *Ephoron* which emerge after dark, immediately mate and die before morning. In a sense this form is almost similar to a cave animal since the nymph is a burrower and thus is not exposed to the light, and since the subimago and imago never see the light.

2. Many species of the genera *Paraleptophlebia, Habrophlebia, Baetis, Cloeon, Pseudocloeon,* and *Centroptilum* engage in nuptial flight during the day. Individuals of these genera are small. They have transparent legs, wings, and caudal appendages in both sexes, and also the abdominal segments 2-6 in the males are transparent. Anyone who has observed these species in nuptial flight knows how extremely difficult it is to see the male individuals in flight. The females are somewhat easier to locate because of their dark abdomens. The

latter, however, enter the nuptial flight only long enough to copulate with a male and then immediately leave to lay their eggs. Thus they are exposed only a short time to their predatory enemies, of which the insectivorous birds are unquestionably the most dangerous.

In attempting to analyze the causes concerned with the tendency for early season species to be dark and their later relatives to be light, *Stenonema tripunctata* Bks. furnishes us an excellent clue. This species spreads its emergence period over several months, starting in late April and extending into September. The first individuals to emerge are very dark all over, while those that emerge in August are much lighter. In fact, some of the latter are almost white. The only answer that seems feasible is that those forms which reach maturity in the spring while the water temperatures are low have more melanin deposited in their chitin and epidermis than those that reach maturity while the water temperatures are higher. This is in accord with the known facts that the optimum temperature for the formation of melanin from tyrosin is 10° C. Thus, in the various series discussed above, it may simply mean that those species that develop under colder temperatures tend to deposit more melanin in the chitin and the underlying tissues of the imago than do those that develop under warmer conditions.

The really crucial question is what makes it possible for one species to reach maturity while the water temperature is low, while the nymphs of another species living in the same stream and under the same environmental conditions must await warmer weather before they can reach maturity. The dark pigmentation of the early forms unquestionably seems to be of some survival value to the individuals, since they emerge and engage in the nuptial dance during the day and are thus exposed to the major enemies of all mayflies, i.e., birds, dragonflies, and a few other carnivores. Under such conditions, light colored individuals would be at a considerable disadvantage. When the lighter colored species do emerge, it is invariably at warmer periods and the individuals emerge mainly at night and engage in the nuptial dance at twilight. It does not seem logical to assume a direct causal connection between the dark coloration as a protective adaptation and time of emergence. All of the early emerging species belong to specialized genera. The more primitive genera emerge later in the season. The various early emergents must therefore possess a physiological make-up that will allow the nymph to reach maturity while the water temperatures are low. Development under a lower temperature in turn probably facilitates the deposition of dark pigments in the resultant imago, and merely as a secondary result the dark coloration apparently is of survival value. In fact, as is easily observed, any species that emerges during the day, no matter how colored, invariably undergoes considerable decimation by winged enemies as subimagoes leave the water and fly toward the vegetation surrounding the body of water in which they dwelt as nymphs.

In *Baetis* and its relatives and in the majority of the species *Paraleptophlebia* however, with the small size and the distinctive distribution of the dark pigmentation to those parts of the body which are underlaid mainly by muscle tissue, there seems no logical way of explaining the phenomenon except by assuming that the color pattern has arisen as a direct result of its survival value. Should the entire exoskeleton be transparent, then the light colored muscles as well as light colored egg masses of the females would show through and thus render these specimens highly conspicuous. As it is, the wings, legs, caudal filaments and, in the males, abdominal segments 2-6 are transparent and thus in the flying insect almost invisible, while the dark areas, being small, are relatively inconspicuous. It should be noted that despite the fact that many of these species reach maturity in and emerge from warm water, nevertheless many of them have certain areas in which melanin is abundantly deposited.

In conclusion, it might be pointed out that, no matter what the coloration or color pattern of any ephemerid may be, it tends to make the individuals of that species inconspicuous in the particular set of environmental conditions where they normally spend their brief adult life spans.