THE MAYFLY SUBIMAGO

George F. Edmunds, Jr.

Department of Biology, University of Utah, Salt Lake City, Utah 84112

W. P. McCafferty

Department of Entomology, Purdue University, West Lafayette, Indiana 47907

INTRODUCTION

Mayflies are unique among present-day insects because they molt after becoming fully winged; in other words, they have a winged preadult life stage. This is the subimago. Because mayfly metamorphosis includes this unique life stage and is thus different from other existing kinds of insect metamorphosis, entomologists have long pondered the exact nature and role of the subimago. Many of the proposed explanations have been based on little or no data. Is the subimago comparable to the adult of other insects or is it perhaps equivalent to the pupa or even the larva? Does it have a functional role or is it merely a relict of a primitive lifestyle? Why and how in certain groups of mayflies has the subimago evidently replaced the adult? Here we review the historical thinking, synthesize the pertinent aspects of available data with considerable unpublished data, and draw conclusions about the function and evolution of the subimago.

CHARACTERISTICS OF THE SUBIMAGO

The subimago of a mayfly is vastly different from the larva, whereas the subimago and adult mayflies are quite similar and in a few species can be difficult to tell apart. There is now little doubt that the subimago and adult represent two separate instars. In early publications, Lameere (40) and Needham et al (52) argued that the change from subimago to adult was only a delamination of the outer layer of cuticle. This idea was refuted by Ide (29), who showed critical cuticular differences as well as significant dimensional

509

differences of some structures in the two forms, and by Taylor & Richards (73), who showed that complete cuticles exist in both forms and result from complete molting cycles that include apolysis and ecdysis.

All male mayflies and most females molt from subimago to adult. However, females of at least two species of the family Leptophlebiidae have an adult stage but do not complete the final molt from the subimago (12, 20). In other words, they become pharate adults, since subimago-adult apolysis occurs but ecdysis does not. In addition, females of a few specialized species have lost the adult stage; there is no molt of the subimago and no pharate adult within the subimago (no subimago-adult apolysis).

Hinton (26) considered the final stage of the mayfly as the adult regardless of whether or not it was preceded by another winged instar, and therefore maintained that it was the subimago that was lost in the specialized females mentioned above. We do not agree because the female subimagos of these mayflies are structurally homologous with subimagos of all other mayflies, and when compared to males of their own species they are similar to the male subimagos, not the male adults.

The winged stages are easily distinguished from each other in the vast majority of mayfly species. Subimagos generally have dull, opaque to translucent wings, and adults have shiny, transparent wings. The outer and hind edges of subimaginal wings are fringed with a row of fine cilia (Figure 1A), and their surface is covered with falciform microtrichia (Figure 1A, B). Adult wings of most species lack cilia, and all lack surface microtrichia (Figure 1C). The body surface of the subimago is more or less covered with microtrichia or microspines, and the caudal filaments are clothed with small hairs (Figure 1D). Except for scattered hairs or microtrichia on the tails of some species, almost all adults lack coverings of the body surface and appear glossy. The forelegs and caudal filaments of subimagos are shorter than in adults, especially in males, and the male genitalia and sometimes eyes are not yet full size.

Although there are certain exceptions to the differences cited above, examination of over 150 genera representing all families showed microtrichia always present on the membrane of subimaginal wings and absent on the membrane of adult wings (G. F. Edmunds, Jr. & W. P. McCafferty, unpublished). In specialized mayflies that have only one winged instar in the female, that instar possesses typical subimaginal microtrichia. Mayflies of the subfamily Oligoneuriinae (family Oligoneuriidae) are peculiar because at molting to adult they shed the subimaginal cuticle from the body but retain the subimaginal cuticle on the wings (13, 14, 20, 55).

Subimagos tend to be slow fliers with little agility in comparison with adults of the same species. Needham et al (52) suggested that the smoother,
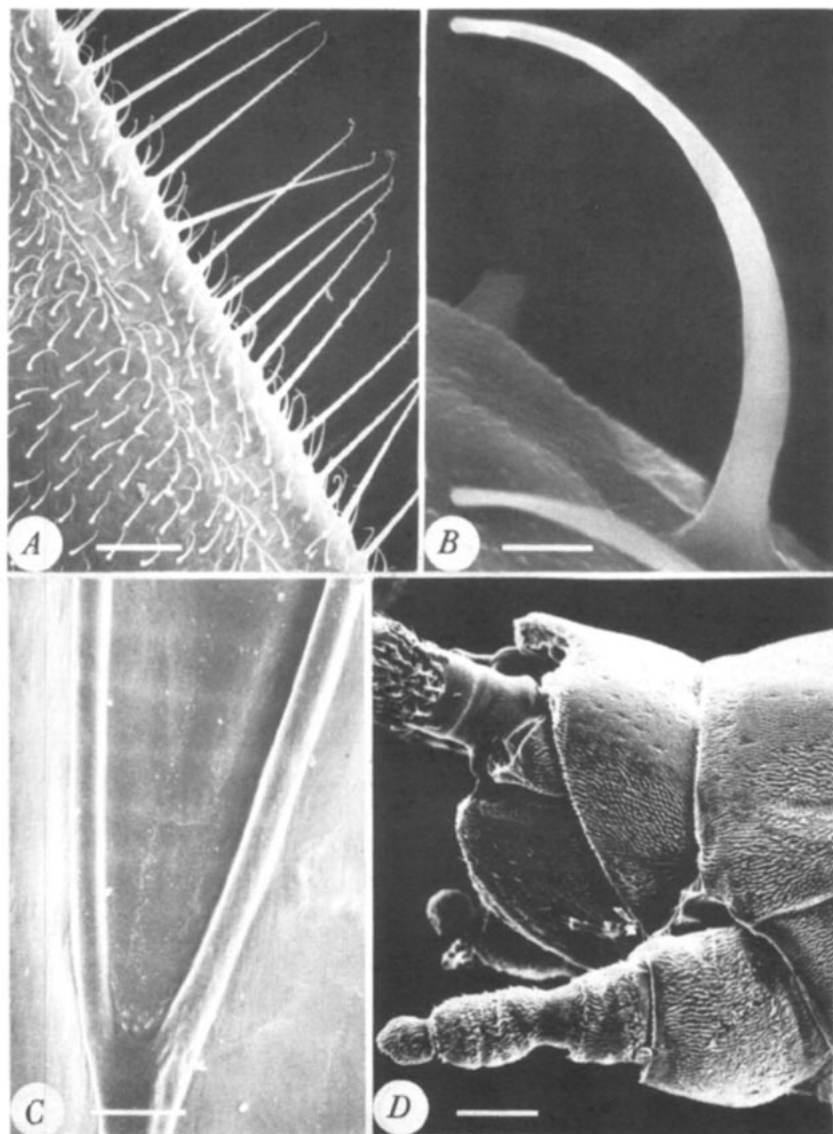
Figure 1 Scanning electron micrographs: (*A*) *Siphlonurus occidentalis* subimago; part of wing surface and margin, ventral view. Note marginal cilia and surface falcate microtrichia. *Scale bar* = 25 μm. (*B*) *Dolania americana* subimago; falcate microtrichia on wing surface. *Scale bar* = 2 μm. (*C*) *Callibaetis ferrugineus* adult; wing surface at fork of radius vein, showing smooth surface, ventral view. *Scale bar* = 50 μm. (*D*) *C. ferrugineus* subimago; apex of abdomen showing surface setae, microtrichia, and microspines, lateral view. *Scale bar* = 125 μm.

hairless surface of the adult would be more favored in flight because of reduced air friction; likewise, Ide (29) suggested that losing the subimaginal covering was an advantage in nuptial flight. We do not agree that wing surface microtrichia necessarily have an adverse effect on flying, particularly in light of the fact that such structures are found on the wings of many insect groups (4) and may actually aid in flight for some (79). Moreover, those oligoneuriine mayflies that retain the subimaginal cuticle on the adult wings are among the fastest flying mayflies.

Weight could influence flying ability. Needham et al (52) reported a distinct loss in weight from the subimago to adult (a 21.5% difference in the species studied, 1.5% due to loss of cuticle and the remainder due to loss of water). Burks (6) stated that adults weighed 25% less than subimagos. Flight differences may be related to the amount of gas held within the body. Pickles (57) found that when the subimago first emerges from the larval skin its large midgut is highly contracted and devoid of gas; indeed its entire body is largely devoid of gas owing to the external pressure exerted on the pharate subimago before it escapes the larval exoskeleton. Adults, on the other hand, were always found to have highly distended midguts filled with air. This inflation occurs later in the life of the subimago or is completed after the molt to adult. Most subimagos are observed flying just long enough to find a resting place after molting from the larva, but prior to gaining the aerostatic advantage of the adults (W. P. McCafferty, unpublished).

Adults can be mistakenly identified as subimagos when molting has not been observed and the adult either retains some of the usual subimaginal features or has features that mimic those of the subimago. One case in point involves species of the subfamily Palingeniinae (all Old World members of the Palingeniidae). Male adults have wings that appear to be subimaginal wings; their wings are even shriveled and twisted on dry-preserved specimens, as is typical of subimaginal wings. In addition, their body and legs are of various dull colors, and their caudal filaments are densely clothed with hair (Figure 2C). Since most specialists, even today, would likely regard such specimens as subimagos, it is not surprising that many early investigators (11, 29, 40, 52, 73) stated that the male and female of *Palingenia longicauda* mated and died as subimagos. Others (6, 15, 60, 76) have erroneously reported that *Plethogenesia,* another palingeniine genus, did not possess an adult stage in either sex.

Morphological examination has indicated that males of all genera of Palingeniinae molt and become adults (G. F. Edmunds, Jr., unpublished). Although females of only three species of these mayflies have been examined (*Palingenia longicauda, Palingenia fuliginosa,* and *Plethogenesia papuana*), we suspect that these and all other females in the group have lost the adult stage.
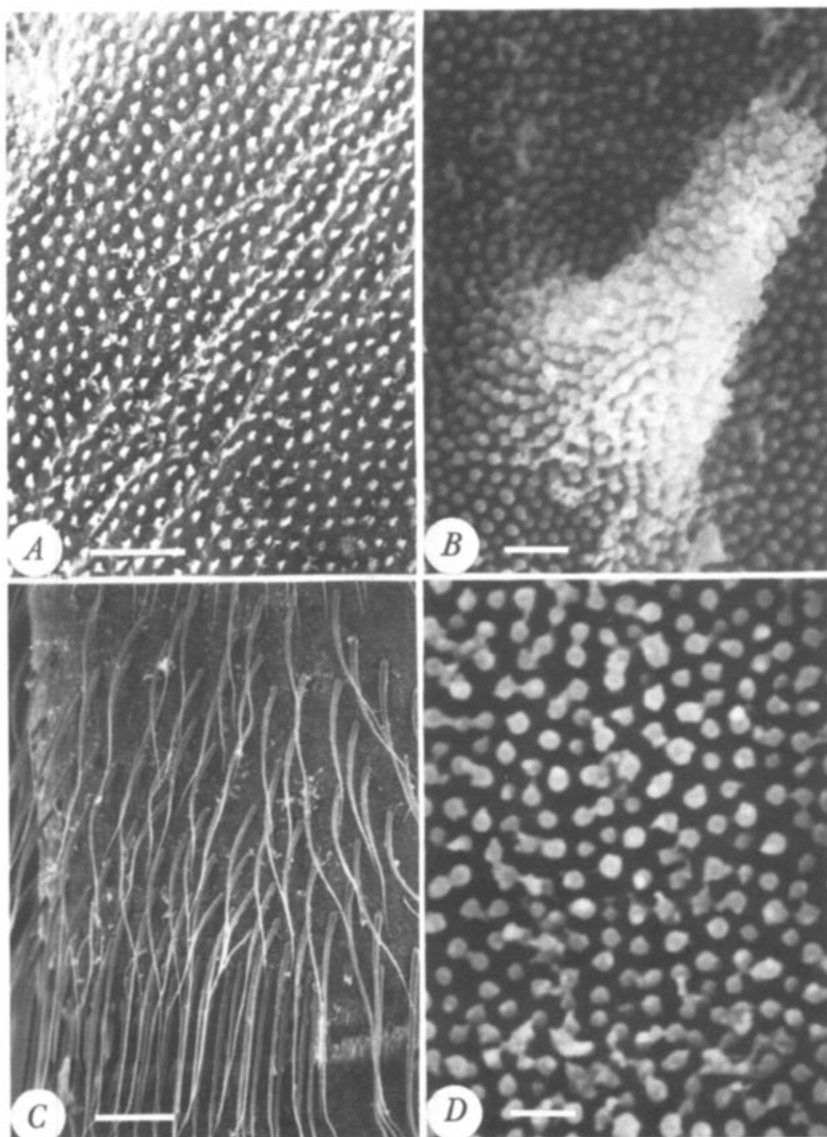
Figure 2 Scanning electron micrographs: (*A*) *Palingenia fuliginosa* adult; large tubercles on wing surface, ventral view. *Scale bar* = 50 μm. (*B*) *P. fuliginosa* adult; large tubercle, enlarged. Note small tubercles on entire wing surface, ventral view. *Scale bar* = 1 μm. (*C*) *P. fuliginosa* adult; setae midway along a tail filament. *Scale bar* = 125 μm. (*D*) *Tricorythus* sp. (sensu lato) adult; small tubercles on wing surface. *Scale bar* = 0.5 μm.

Male adults differ from the subimagos in that some body parts, e.g. the upper thorax, are more glossy. The adult wings have comparatively few fringe cilia and, as is consistent for all mayflies, they lack wing surface microtrichia. The surface is instead granular (Figure 2A, B), and this quality imparts the subimaginal appearance to the wings.

Field observations substantiate our findings. Swammerdam (71), who based his pioneering work in insect anatomy in part on *P. longicauda,* had previously reported that males of that species molt twice but females only once. Russev (61; personal communication) and Soldán (66; personal communication) have indicated that males of *Palingenia* are typical of other mayflies in being agile in flight as adults and clumsy as subimagos.

ORIGIN OF THE SUBIMAGO

Workers have debated whether the subimago was primitive or specialized. Kimmins (32) believed that the subimago was a specialized stage, but others (3, 8, 24, 58, 62, 64) believed it was a retained primitive stage. These ideas were essentially based on indirect evidence. However, Kukalova-Peck (37, 38; personal communication) has presented the following convincing fossil data in support of a primitive existence of the subimago.

In early fossil Ephemeroptera or Ephemeroptera precursors and in the extinct orders Protodonata, Diaphanopteroidea, Paleodictyoptera, and Megasecoptera, the developing wings of immature forms were freely articulated with the thorax, and wing development proceeded gradually through numerous molts. Further along in the evolution of these lineages, which included certain Paleozoic mayflies, developing wings became fused to the thorax in young larvae, and articulation occurred at a later time in the life cycle, so there were a number of subsequent instars with incompletely developed but articulated wings. Kukalova-Peck referred to all of these preadult instars with articulated wings as subimagos and believed that some may have been capable of flight.

The paleontological evidence suggests that the fully winged subimago of primitive fossil mayflies as well as certain extinct paleopterous groups and the subimago of modern mayflies are homologous. As an apparent derivation from the more primitive types of metamorphosis involving several subimaginal instars, metamorphosis in modern mayflies involves a large number of larval instars (with fused wing pads) but only one instar referable to the subimago.

METAMORPHOSIS

Considerable structural transformation occurs in the life of most aquatic insects and reflects a dramatic shift from an aquatic to terrestrial habitat. Thus

in mayflies, the greatest change occurs at the molt from larva to subimago. Therefore, although in entomology the term "emergence" generally refers to the molt to adult, with reference to mayflies we use this term for the molt to subimago.

Transformation in mayflies involves the maturation of adult features by progressive formation of exclusively adult structures (e.g. reproductive organs) and by change in the form of certain larval and subimaginal structures (e.g. eyes). In addition, exclusively larval features are eliminated or radically reduced (e.g. mouthparts and digestive system). Maturation may commence at various times during the larval period but in large part occurs in the later instars or last instar. Although most adult structures are apparently preformed by the end of the larval stage, including restored wing articulation in the contained pharate subimago, such structures do not greatly affect larval form because most are packaged within the larval cuticle. Subtle features such as dark wing pads and opaque eyes distinguish the final larval instar from earlier instars (59). Reduction and loss of larval features occur essentially at the molt to subimago. However, further reduction or complete loss of certain features such as mouthparts (29) or, in some species, gills (70) takes place at the adult molt. Cellular processes preceding atrophy begin in the larval stage (57), and some atrophy is already apparent in the mature larvae of a few species (e.g. 69).

A distinction must be made between the maturation of a structure and the stretching, expansion, or unfolding of a preformed structure into its adult state. For example, in the mature larva the articulated subimaginal wing, and within it the adult wing, are already preformed but folded within a small casing. Ide (29) studied structural transformation in *Ephoron leukon*. He noted significant change in the male forelegs, tails, and genitalia. The foreleg tarsi of the adult male are 5–7 times their larval length. Although the adult leg is preformed within the mature larva, almost all of its expansion takes place at the molt to adult, since it fits inside the leg of the subimago folded like the bellows of an accordian (Figure 3).

Soldán (68) found subimaginal and adult features of *Palingenia fuliginosa* preformed within the mature larva. Edmunds determined that male adult foreleg tarsi are about 8.5 times their larval length, with almost all of the expansion taking place at the molt to subimago. He also found that male tails expand to 5 times the larval length, but with about half the expansion at the molt to subimago and half at the molt to adult (G. F. Edmunds, Jr., unpublished).

It appears that structural expansion is variously apportioned to the subimaginal and adult molts in mayflies. *Nixe criddlei* tarsi are 1.83 times longer than those of the larva in the subimago and 2.61 times longer than those of the larva in the adult; *Siphlonurus occidentalis* tarsi are 2.0 and 2.9 times
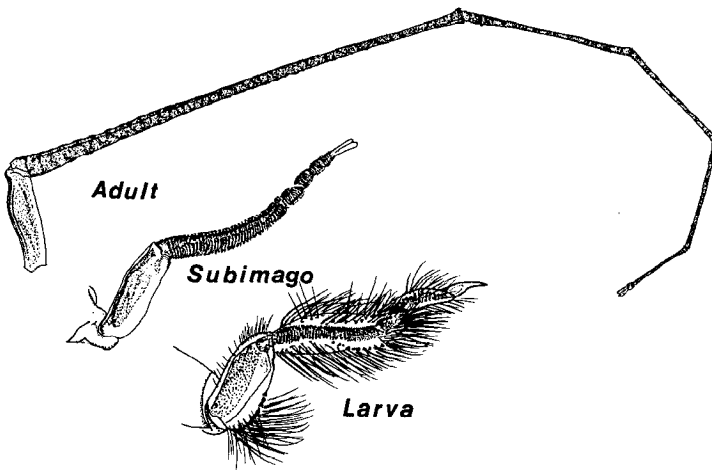
Figure 3 Foreleg of male larva, subimago, and adult of *Ephoron album* at same scale, demonstrating expansion, changes of proportions, and length through two molts.

the larval length in the subimago and adult, respectively; and the tarsi of *Ametropus ammophilus,* whose larval forelegs are relatively short compared with those of other mayflies, are 2.15 and 4.98 times the larval length in the subimago and adult, respectively (G. F. Edmunds, Jr., unpublished).

Transformations are not as extreme in female mayflies as they are in males. This is because some degree of change in proportion is related to the secondary sexual features characteristic only of male adults. Males locate female mates visually and grasp females with their forelegs during mating. Thus, the males of most species have much larger eyes and longer forelegs.

LONGEVITY AND REPRODUCTIVE MATURITY

Mayflies, with few exceptions, exhibit either of two basic patterns in longevity of the winged stages. The longer and more predominant of the two patterns is the more ancestral pattern; it includes a subimaginal period of usually 8 hr to 2 days (rarely 3 days) and an adult period of about 1 day to 2 wk or more (but rarely beyond 3 days).

The second and shorter of the two basic longevity patterns evolved independently in at least nine derived lineages of mayflies (17). (See References 16 and 48 for evolutionary relationships of major lineages.) This shorter pattern includes a male subimaginal period of a few minutes and a male adult period of a few hours at most. Female longevity is similar to that of the male except in four of these derived lineages, wherein the female adult has been eliminated entirely and the longevity of the female subimago has become

roughly equivalent to that of the male adult. The four highly specialized lineages are represented by (a) *Ephoron, Tortopus,* and *Campsurus;* (b) *Behningia* and *Dolania;* (c) Prosopistomatidae; and (d) Palingeniinae. A somewhat intermediate deviation from these patterns is demonstrated by *Tricorythodes atratus* (21) and *Tricorythodes minutus* (G. F. Edmunds, Jr., unpublished). The males emerge after dark and the females emerge at low light early the next morning; mating occurs at 8:00–10:00 AM the same day the females emerge.

In the specialized lineages that have lost the female adult, the emergence of male subimagos of *Ephoron album* was found to occur 5–25 min earlier than that of female subimagos (W. P. McCafferty, unpublished), and male subimagos appeared an average of 32.5 min earlier in *Dolania americana* (56). This emergence behavior presumably allows the male adults and the reproductive female subimagos to occur simultaneously within the very brief winged-stage longevity pattern. Emergence of the subimago evidently occurs at about the same time in the two sexes of most species that have the longer longevity pattern (e.g. 5, 10, 77), but various degrees of asynchronous emergence of the sexes are known in some species (e.g. 5, 19, 22, 34).

The exact subimaginal period within an observed range of longevity for any one species may be related to certain environmental factors. Relative humidity is a critical factor for the subimago; very high or, more often, low relative humidity results in death or unsuccessful molting (6, 21; W. P. McCafferty & A. V. Provonsha, unpublished). In at least some species this molt occurs relatively early at higher temperatures, and there is a threshold that must be reached before molting proceeds (42; J. G. Peters & W. L. Peters, personal communication).

Regarding differential longevity, Wesenberg-Lund (80) theorized that mayflies that have a very brief longevity pattern and that mate soon after emergence differ from the longer lived mayflies in already having mature ovaries and eggs in the full-grown larva. Koss (36) maintained that eggs are fully formed in the larvae of all mayflies, since he consistently could find no external differences in eggs taken from mature larvae, subimagos, and adults of the same species. Soldán (67) showed that in six diverse species with winged stages of long duration, oogenesis begins in early instars of female larvae and eggs are formed prior to emergence of the subimago. Extraneous differences in the eggs of subimagos and adults of some *Baetis* species have been reported (35), but it is not known if these differences affect egg maturity or potential for fertility (R. D. Waltz, personal communication).

We assume from the available data that there is no correlation between the duration of winged stages in mayflies and the completion of oogenesis. If this assumption is correct, all female mayflies may be physiologically, if not behaviorally, capable of reproduction as subimagos. Besides the fact that

females of certain species do reproduce as subimagos when they lack the adult stage, other supporting evidence includes a report of mating and successful fertilization of eggs in female subimagos of three species whose females do molt to adults (12) and perhaps the occasional observation that adult males mate with female subimagos immediately upon their emergence (18). In the laboratory, artificially inseminated female subimagos of *Stenacron* and *Stenonema* did not oviposit until they had molted to adults (W. P. McCafferty, unpublished).

Soldán (67) found that spermatogenesis begins in early instars and that sperm are mature in the last larval instar of males of the six species he studied. Spermatozoa are already in place in the seminal vesicles of subimagos (41). Mating by male subimagos, however, has never been reported, even though these males probably contain mature gametes; this is not surprising to us because they generally lack the fully developed external genitalia. Levy (41) found that even though the male reproductive systems of the seven species he studied were present in mature larvae, abrupt realignments of internal structures as well as a slight increase in certain associated muscles were not completed until the adult molt. In addition, male subimagos, at least initially upon emergence, generally lack the flying agility of the adults, which we feel is required for a high degree of success in capturing flying female mates.

HABITAT TRANSITION

The closest coordination of habitat transition and metamorphic transformation in aquatic insects is found in those insects that transform from aquatic larvae to winged terrestrial forms at approximately the air/water interface (47). Somewhat less coordinated methods involve either exiting from the water at an aquatic stage and then immediately commencing transformation, or transforming to the terrestrial stage under water and then immediately exiting from the water (47). Each of the three methods of habitat transition is found among mayflies.

The habitat transition of *Hexagenia bilineata* has been studied in detail (W. P. McCafferty, unpublished) and illustrates the general process of surface emergence among mayflies, although there are slight variations among different species. (See Reference 57 for comparative details of a species that emerges on land.) Larvae swim or float to the water surface and slightly expose the dorsum of the thorax. A gaseous layer that forms between the larval cuticle and the pharate subimago provides buoyancy and is evidenced by several small extruding bubbles. During ecdysis nearly the full length of the legs, which are held straight against the underside of the body at the point of escape from the larval cuticle, comes in contact with water. At the termination of ecdysis the subimago rests briefly before taking flight, with at

least the middle and hind legs and part of the underside of the abdomen on the water. The initial downstrokes of the subimaginal wings often bring them in contact with the water.

Most mayflies are surface emergers and crawl or swim/float to the surface as larvae. Both means of surfacing are found in some species (2, 33, 54, 63); this is particularly common in Baetidae and Ephemerellidae (W. P. McCafferty, unpublished). Some species crawl partially out of the water, e.g. certain species of *Leptophlebia* (33, 50, 75) and *Ameletus* (T. J. Fink, unpublished).

Complete water exiting by the larva occurs generally in the family Siphlonuridae (sensu 48), with few exceptions (7), and in a few other mayflies [some *Paraleptophlebia* (G. F. Edmunds, Jr., unpublished), several Oligoneuriidae, and the Baetiscidae]. These larvae generally crawl up to several centimeters from the water; however, larvae of Baetiscidae may crawl 1 m or more from the water (D. W. Bloodgood, personal communication).

Dual emergence behavior is found in primitive members of the Oligoneuriidae. Morgan (50) noted that *Isonychia* larvae would crawl out on shore prior to emergence and that subimagos would also emerge at the surface midstream. Subimagos of *Isonychia* emerge at the surface of deep and swift water (46), but larvae crawl out of shallow, calmer water. Dual behavior has also been observed in *Coloburiscus,* another oligoneuriid (81).

Complete underwater emergence has been observed in certain species within seven genera in four families (21, 27, 28, 30, 31, 49), including *Nixe* (G. F. Edmunds, Jr., unpublished). Underwater emergence behavior varies among species, with the subimago emerging at different depths and either crawling or floating to the surface. Kimmins (31) found that individuals would alternatively emerge at the surface if larvae lost their underwater foothold. We expect that underwater emergence probably occurs in other mayflies, especially night emergers. Also, it is feasible that some mayflies that have been reported as surface emergers might actually emerge just below the surface, as is common in some caddisflies (39).

The time from the splitting of the larval thoracic cuticle to subimaginal flight varies considerably (1, 2, 54). The period can last up to 15 min or more in the Siphlonuridae and up to 10 min in the Baetiscidae; mayflies of both families exit from the water as larvae. The period is apparently much shorter for species that emerge at the surface or under water. However, precise data on this subject are rare. The period ranges from 10 to 20 sec in *Hexagenia bilineata,* but the subimago appears to take flight instantaneously in some species (W. P. McCafferty, unpublished).

Habitat transition and emergence are precarious events for aquatic insects (47). Subimagos are highly vulnerable to predation by surface-feeding fish (e.g. 23, 44, 72) or to terrestrial predation if they emerge on land (e.g. 50, 53). The initial flight of the subimago may signal such flying predators as

Odonata (46), birds (e.g. 52), and bats (G. F. Edmunds, Jr., unpublished; R. M. Timm, personal communication). A common additional problem for many species involves the sloughing of the larval exuvia. When either the wings or caudal filaments are not freed the subimago does not survive (W. P. McCafferty, unpublished).

Water represents perhaps the most serious potential hazard to the emerging subimago because if it gets wet, it can easily become entrapped. Since emerging subimagos of most mayflies contact water, a few emerge under water, and all have potential for contacting water, the fact that they generally do not get wet suggests that subimagos are hydrofuge. Hydrofuge properties appear to be correlated with the various styles of habitat transition discussed above.

HYDROFUGE PROPERTIES

Ide (29) stated that the hairy surface of the body, legs, and wings of the subimago would allow the mayfly to overcome the hazards of emergence. Wesenberg-Lund (80) came to the same conclusion; he emphasized the importance of the wings of the subimago. Dense coverings of small hairs or scales on the cuticle of many aquatic and semiaquatic insects are known to bestow a hydrofuge quality to the associated area of cuticle (e.g. 25, 74, 78) with various functions among different aquatic insects (47). Thus, the presence of cuticular microtrichia in all subimagos and the absence of them in adults of most species suggests that the subimago is a much more effective hydrofuge form than the adult. This is further suggested by the association of the subimago with water in habitat transition and by field observations indicating that adults are not generally hydrofuge. For example, the female adults of certain species of *Baetis* actually crawl into the water to oviposit; owing to their small size and weak legs, this would probably be impossible if they were hydrofuge. Peters & Peters (56) reported that subimagos of *Dolania* can resume flight after contacting water but that adults are unable to leave the water if contact is made. Also, the wings of stonefly adults, which have the same surface microtrichia as wings of subimagos (4), are strongly hydrofuge when individuals are blown onto the water (W. P. McCafferty, unpublished).

Laboratory or field observations of the relative hydrofuge nature of the subimago versus the adult were made for a number of different North American species in the families Siphlonuridae, Baetidae, Heptageniidae, Leptophlebiidae, Ephemerellidae, and Tricorythidae (G. F. Edmunds, Jr. & T. J. Fink, unpublished). The method of observation was to gently toss individuals onto the water and then compare their ability to escape the water surface. Ten or more individuals of both subimagos and adults of each species were

observed. Without exception, subimagos escaped more ably than adults. This is highly suggestive of differences in the hydrofuge quality of the two stages, but a strict conclusion cannot be drawn from these simple observations alone since the differences could be due entirely to behavioral responses.

We agree with the conclusions of Ide (29) and Wesenberg-Lund (80) concerning the role of a hydrofuge subimago in habitat transition. If sub-imaginal surface structures are hydrofuge then one should expect that a similar protection from water hazard would also be found in the few mayfly adults that possess apparent hydrofuge surface areas. Adults of certain species have profuse hairs or microtrichia on their tails, including male *Behningia* and Palingeniinae and male and female *Siphloplecton* and Tricorythinae (G. F. Edmunds, Jr. & W. P. McCafferty, unpublished). Individuals of these same groups fly very close to or skim the water surface. Male adults of *Prosopistoma* and female adults of *Caenis* also have what appear to be hydrofuge tails, but we can only surmise that they come into close association with water because details of their behavior remain unknown. Cilia along the edge of adult wings of certain species with very small individuals do not appear to have a hydrofuge function, but rather seem to be related to flight.

Adults of some mayflies have atypical flight behavior that increases their risk of contacting the water surface; they possess wings that are apparently hydrofuge. Some such adults belong to the subfamily Oligoneuriinae. *Lachlania* adults, for example, fly at speeds comparable to those of dragonflies only about an inch above the water, often following the pattern of splashing riffles (14, 15). Exuviation in this group takes place in flight or on the water, and as mentioned previously, the subimaginal cuticle is shed everywhere but on the wings. Thus, some hydrofuge properties of the subimago are retained in the adult.

The Palingeniinae demonstrate an additional mechanism of incorporating hydrofuge properties into the adult wings, and in fact over most of the adult body. Females of this group remain subimagos, and the males, although they transform to the adult, are very subimagolike and often mistaken for sub-imagos. Mating behavior has been reported (e.g. 13, 51, 61), and mating of *Palingenia fuliginosa* has been strikingly presented in the film *Ephemera* (40a). These insects swarm while skimming and regularly contacting the water, and mating can take place on the water surface. Adults, however, easily resume flight after contacting water.

The surface of the adult wings of Palingeniinae (Figure 2*A*, *B*) (G. F. Edmunds, Jr. & W. P. McCafferty unpublished) has coarse granules which are ~2.5 μm in diameter in *P. fuliginosa* and which appear as dense bundles of smaller granules. The coarse granules correspond in position to the sub-imaginal microtrichia, as evidenced by the very rare presence in *P. fuliginosa* of an occasional microtrichia, always originating in a coarse granule (W. P.

McCafferty, unpublished). Spaces between the coarse granules are densely covered with smaller granules, ~0.2 μm in diameter in *P. fuliginosa.* Presumably it is this granular sculpturing on the wings and other parts of the body that not only makes these adults appear subimagolike but also makes them hydrofuge. Textured, semiopaque wings have also evolved independently in adults of the Tricorythinae (Figure 2D) (G. F. Edmunds, Jr., unpublished), and as mentioned before, adults of this group skim the water.

The speed with which subimagos were able to right themselves after being placed on their side with wings contacting water varied immensely among subimagos of different genera observed (G. F. Edmunds, Jr. & T. J. Fink, unpublished). The results could be based on differences in hydrofuge capacity, differences in behavioral response among mayflies, or both. Siphlonurids *(Siphlonurus, Ameletus, Parameletus)* were slow to respond, and most of the individuals, especially of *Siphlonurus,* were entrapped on the surface. Heptageniids *(Stenacron, Nixe, Heptagenia)* righted themselves faster than the siphlonurids and almost always escaped successfully. Leptophlebiids *(Paraleptophlebia)* and ephemerellids *(Ephemerella, Drunella)* responded rapidly and escaped so fast that sometimes it was impossible to see if they righted themselves before taking flight.

CONCLUSIONS AND THEORY

Although the adult mayfly may be homologous with the adults of other extant insects, it is possible that the adults of some insects are actually homologous with the subimago of mayflies and extinct orders. Taylor & Richards (73) reasoned that the mayfly subimago was homologous with adults of other insects because the adult of the mayfly was devoid of surface hairs and microtrichia like postadult instars that result from hormonally induced molting of some other adult insects, such as moths. They also reasoned that the adult mayfly was a specialized postadult instar, an idea that had previously been put forth by Lameere (40) and Needham et al (52). Although we agree that mayfly subimagos may be homologous with the adults of some other pterygote groups, available data do not support the idea that the ultimate mayfly instar is a specialized postadult instar. We suggest, instead, that if such homologies exist, then the pertinent lineages of neopterous insects have lost the original, true adult instar and their reproductive functions have been incorporated into a former preadult (subimaginal) instar. Apomorphic tendencies apparent within Ephemeroptera suggest this mode of evolution.

Maiorana (43) hypothesized that the function of the subimago was to allow necessary growth from the larval form to the adult form that could not otherwise be accomplished in a single molt. She thus considered the mayfly subimago to have a metamorphic transformation function similar to that of a

holometabolous pupa. Our data and others indicate that growth of almost all adult structures occurs before the subimaginal molt, but that full expansion or unfolding of structures such as long forelegs or caudal filaments may not be completed until the adult molt. The relegation of full appendage elongation to the adult molt of males of most species could be due to a differential selective advantage in the two stages. For example, in the male adult very long forelegs may be necessary for grasping the female, but they may be a hindrance or of no value to the male subimago. In the case of females, the subimago appears to be reproductively mature, and there is little if any structural expansion from the subimago to the adult. In a sense, the female adult stage may be superfluous, and in fact it has been lost independently in at least four different lineages of mayflies.

The winged stages of mayflies have become extremely short lived (a few hours at most) in several groups of mayflies. According to current theories about the evolutionary relationships of mayfly families and subfamilies, this short longevity pattern appears to have evolved independently in nine different lineages. This indicates some repeated trends in mayflies toward minimizing the terrestrial portion of the life cycle, a portion that in modern-day mayflies is already dedicated exclusively to propagation. A possible consequence of this tendency might be the loss of the adult stage, at least in the female, since the four lineages where this has occurred are in fact among these nine specialized lineages. The adult has been maintained in the males of all of these lineages and the longevity of the male subimago has been reduced to a few minutes, which is perhaps the minimum period required for completing the molting process. There appears to be no correlation between the longevity of winged stages and the onset of adult maturation processes in the larva.

Snodgrass (65) and Schaefer (62) considered the subimago to be a relict with no adaptive function for the modern-day mayfly. To the contrary, new data clearly show that the subimago is hydrofuge and that the mayfly requires this hydrofuge quality to make the usual hazardous habitat transition from water to land without becoming easily entrapped by the water surface. Underwater emergence requires that the subimago be hydrofuge.

The relatively few adults that closely resemble subimagos, such as the males of Palingeniinae, possess body-surface structures and sculpturing that appear to make them hydrofuge. Behavior in these forms is atypical in that the adults contact water during swarming and mating.

The hydrofuge function of the subimago is undoubtedly important to mayflies, but whether the hydrofuge wings of the subimago originally evolved as an adaptation for preventing water entrapment or instead were already available before subimagos needed to contend with water entrapment is another question. This question is pursued because the wing membranes of several other insect orders possess similar surface microstructures although

they are not necessarily involved in making a habitat transition or subjected to the hazards of water (4).

Insect wings that possess surface membrane microstructures are folded up within a sheath or wing casing prior to emergence in mayfly and stonefly larvae and in the pupae of such higher insects as wasps and flies. Paurometabolous insects whose wings are not encased in a small sheath or pad but grow as progressively expanding external structures do not have such microstructures covering the membranes of their adult wings. Thus there appears to be a positive correlation between wings that must unfurl or unfold upon emergence and the presence of microtrichia. We speculate that these hydrofuge structures may prevent the membrane of the wing from sticking to itself in the folded, furled, or convoluted position and may thus facilitate unfolding at emergence. Microtrichia on other body parts as well as the wings could also feasibly facilitate sloughing of the larval cuticle or general exuviation. The Odonata are exceptional in that their wings develop in sheaths but lack microtrichia on the membranes. The correlation suggested may still hold, however, because dragonflies slowly pump their wings up at emergence, perhaps obviating the need for microtrichia. In addition, the possible independent origin of Odonata wings, as evidenced by numerous differences from other modern pterygotes (45), may explain this apparent anomaly.

Several facts favor a theory that the surface microtrichia of the wings of subimagos were not originally associated with hydrofuge function, but evolved either in relation to exuviation or wing unfurling or for some other reason. The Siphlonuridae, the most ancestral family of modern-day mayflies, emerge on land, which suggests that the most primitive method of habitat transition involved exit from the water by larvae. It is questionable whether occasional falls into water by land-emerging subimagos would have been a strong or frequent enough selection pressure to have favored the evolutionary development of microtrichia. Subimagos of Siphlonuridae are very slow to respond to placement on water, which supports the contention that this is an infrequent occurrence since a strong escape response is not present. Penniket (53) noted that subimagos of these primitive mayflies tend to walk about rather than fly and also to take a strong gripping foothold if threatened. This behavior is not typical of other mayflies.

Dual emergence behavior, i.e. emergence either on land or at the water surface, is found in ancestral species of Oligoneuriidae, a family closely related to Siphlonuridae (48). Also, species apparently belonging to the more primitive genera of Leptophlebiidae have been observed to crawl partially or completely out of the water to emerge. Surface or underwater emergence exists in all other mayflies except for the unusual and highly derived Baetiscidae. A possible reason for this apparent secondary instance of land emergence may be the insects' molting from an unusually thick larval cuticle.

Surface emergence would theoretically be possible once the subimago was preadapted, or exaptated, to being unwettable because of surface microtrichia. Two situations in the evolution of mayflies could have been related to a shift from an ancestral land emergence to surface emergence, which is now by far the predominant behavior. An increase in predatory pressure on land could have favored surface emergence. A consideration of the kinds of habitat transitions among the families of mayflies, in light of their relationships and principal aquatic habitats, however, strongly suggests that the behavioral shift was related to a shift from primarily still- or quiet-water habitats to running-water habitats where larval exit from the water would be problematic. Whereas many species of Siphlonuridae remain inhabitants of still-water habitats or quiet edgewaters and pools of streams, at least in later larval life, other mayflies are mainly represented by flowing-water species. At least some members of the Oligoneuriidae can emerge at the water surface or on land. These larvae crawl out on land in quiet edgewaters, but when they emerge from riffles and deeper midstream areas they must do so at the surface. The relationship of habitat and behavior in the Oligoneuriidae may illustrate the general relationship that we have suggested for mayflies, and their optional emergence may represent an evolutionarily intermediate behavior. Partial crawling out of the water to emerge, as in some Leptophlebiidae, may also represent another intermediate type of behavior.

Emergence is generally slow in the land-emerging Siphlonuridae, and is generally much faster in species that emerge at the water surface. Surface emergence may have favored faster emergence, possibly because of the potential for wetting, predatory pressure from surface-feeding fish, and/or the possibility that individuals could be variously displaced by floating downstream for a considerable distance, which might affect eventual mate-finding efficiency. The putatively secondarily derived land emergence of the Baetiscidae is also very slow. We speculate that this is due to the extra difficulty of splitting and escaping from a very thick and armorlike thoracic cuticle. With the evolution of a carapacelike cuticle in this group more emergence time may have been required, and the above disadvantages of slow emergence at the surface of a stream would have favored exit from the water by the larvae.

Although the hydrofuge nature of the subimago would explain why this stage has been retained in mayflies, either in terms of possible exuviation/wing unfurling function or habitat transition, it still remains to be explained why the final molt and adult stage are retained in males despite obvious trends to shorten the terrestrial portion of the mayfly life cycle. Why haven't the habitat transition function plus possible emergence function and reproductive function been generally incorporated into one instar? Why, in particular, do males of Palingeniinae molt from a hydrofuge subimago to a specialized hydrofuge adult? Although all female mayflies are evidently sexually mature

as subimagos and male subimagos possess mature gametes, males evidently are never reproductively mature as subimagos, i.e. male subimagos cannot mate. We have never found fully formed male genitalia, including the female-clasping forceps, in subimagos of any species. The male genitalia grow in a paurometabolous fashion rather than being prepackaged like legs and wings, and this may be a major factor in restricting mating to male adults.

The fact that adult properties have not been incorporated into the male subimago so that the final molt might be eliminated may be due to some incompatability of the hydrofuge function with possible emergence function and the mate-capturing and copulating function. If such incompatibility exists we cannot account for it, particularly in view of some other aquatic insects that combine the roles in the ultimate instar. We must continue to assume that two molts from the larval stage are required for males to attain reproductive maturity and function. Thus the subimago has at least some necessary role in transformation.

ACKNOWLEDGMENTS

Literature Cited

1. Baykova, O. Y. 1974. Discovery of the first males of the genus *Pseudocloeon* (Ephemeroptera) in the USSR. *Entomol. Rev.* 47:320–23

2. Berner, L. 1950. *The Mayflies of Florida.* Gainsville, Fla: Univ. Fla. Press 267 pp.

3. Boas, J. E. V. 1899. Einige Bermerkungen über die Metamorphose der Insekten. *Zool. Jahrb. Syst.* 12:285–416

4. Bocharova-Messner, O. M. 1977. Taxonomic importance of insect wing surface microstructures. Transl. 1978 in *Proc. Acad. Sci. USSR Biol. Sci.* 237:517–20 (From Russian)

5. Boerger, H., Clifford, H. F. 1975. Emergence of mayflies (Ephemeroptera) from a northern brown-water stream of Alberta, Canada. *Verh. Int. Ver. Limnol.* 19:3022–28

6. Burks, B. D. 1953. The mayflies, or Ephemeroptera, of Illinois. *Ill. Nat. Hist. Surv. Bull.* 26:1–216

7. Campbell, I. C. 1986. Life histories of some Australian siphlonurid and oligoneuriid mayflies (Insecta: Ephemeroptera). *Aust. J. Mar. Freshwater Res.* 37:261–88

8. Carpenter, G. H. 1928. *The Biology of Insects.* London: Macmillan. 473 pp.

9. Deleted in proof

10. Clemens, W. A. 1917. An ecological study of the mayfly *Chirotenetes. Univ. Toronto Stud. Biol. Ser.* 17:1–43

11. Cornelius, C. 1848. *Beiträge zur nähren Kenntniss der* Palingenia longicauda

Olivier. Elberfeld, Germany: Büschler. 37 pp.

12. Degrange, C. 1960. Recherches sur la reproduction des Éphéméroptères. *Trav. Lab. Hydrobiol. Piscic. Univ. Grenoble* 50–51:7–193

13. Eaton, A. E. 1883–1888. A revisional monograph of recent Ephemeridae or mayflies. *Trans. Linn. Soc. London 2nd Ser. Zool.* 3:1–352

14. Edmunds, G. F. Jr. 1951. New species of Utah mayflies. I. Oligoneuriidae (Ephemeroptera). *Proc. Entomol. Soc. Wash.* 53:327–31

15. Edmunds, G. F. Jr. 1956. Exuviation of subimaginal Ephemeroptera in flight. *Entomol. News* 67:91–93

16. Edmunds, G. F. Jr. 1972. Biogeography and evolution of Ephemeroptera. *Ann. Rev. Entomol.* 17:21–42

17. Edmunds, G. F. Jr., Edmunds, C. H. 1980. Predation, climate, emergence and mating of mayflies, In *Advances in Ephemeropteran Biology*, ed. J. F. Flannagan, K. E. Marshall, pp. 277–85. New York: Plenum. 552 pp.

18. Edmunds, G. F. Jr., Jensen, S. L., Berner, L. 1976. *The Mayflies of North and Central America*. Minneapolis: Univ. Minnesota Press. 330 pp.

19. Friesen, M. K., Flannagan, J. F., Laufersweiler, P. M. 1980. Diel emergence patterns of some mayflies (Ephemeroptera) of the Roseau River (Manitoba, Canada). See Ref. 17, pp. 287–96

20. Grandi, M. 1947. Contributi allo studio degli "Efemeroidei" Italiani IX. *Oligoneuriella rhenana* Imh. *Boll. Ist. Dei Entomol. Univ. Studi Bologna* 16:176–218

21. Hall, R. J., Berner, L., Cook, E. F. 1975. Observations on the biology of *Tricorythodes atratus* McDunnough (Ephemeroptera: Tricorythidae). *Proc. Entomol. Soc. Wash.* 77:34–49

22. Harper, F., Magnin, E., Harper, P. P. 1983. Diel periodicity of emerging mayflies (Insecta: Ephemeroptera) in a Laurentian stream. *Aquat. Insects* 5:21–31

23. Harris, J. R. 1952. *An Angler's Entomology*. London: Collins. 268 pp.

24. Hinton, H. E. 1963. The origin and function of the pupal stage. *Proc. R. Entomol. Soc. London* 38:77–85

25. Hinton, H. E. 1976. Plastron respiration in bugs and beetles. *Insect Physiol.* 22:1529–50

26. Hinton, H. E. 1981. *Biology of Insect Eggs*, Vol. 2. New York: Pergamon. 778 pp.

27. Hughes, D. 1985. Western march browns. *Fly Fisherman* 16(3):44–47

28. Ide, F. P. 1930. Contributions to the biology of Ontario mayflies with descriptions of new species. *Can. Entomol.* 62:204–13, 218–31

29. Ide, F. P. 1937. The subimago of *Ephoron leukon* Will., and a discussion of the imago instar (Ephem.). *Can. Entomol.* 68:234–38

30. Kennedy, C. H. 1926. The nymph of *Ephemera guttulata* Pictet with notes on the species. *Can. Entomol.* 58:61–63

31. Kimmins, D. E. 1941. Under-water emergence of the subimago of *Heptagenia lateralis* (Curtis) (Ephemeroptera). *Entomologist London* 74:169–70

32. Kimmins, D. E. 1942. Keys to the British species of Ephemeroptera with keys to the genera of the nymphs. *Sci. Publ. Freshwater Biol. Assoc.* 7:1–64

33. Kjellberg, A. G. 1972. Autekologiska studier oever *Leptophlebia vespertina* (Ephemeroptera) i en mindre skogstjarn 1966–1968. *Entomol. Tidskr.* 93:1–29

34. Kondratieff, B. C., Voshell, J. R. 1984. The North and Central American species of *Isonychia* (Ephemeroptera: Oligoneuriidae). *Trans. Am. Entomol. Soc.* 110:129–244

35. Kopelke, J.-P., Müller-Liebenau, I. 1982. Eistrukturen bei Ephemeroptera und deren Bedeutung für die Aufstellung von Artengruppen am Beispiel der Europäischen Arten der Gattung Baetis Leach, 1815 (Insecta: Baetidae). Teil I: *alpinus-*, *lutheri-*, und *lapponicus-*gruppe. *Gewässer Abwässer* 68/69:7–25

36. Koss, R. W. 1968. Morphology and taxonomic use of Ephemeroptera eggs. *Ann. Entomol. Soc. Am.* 61:696–721

37. Kukalova-Peck, J. 1978. Origin and evolution of insect wings and their relation to metamorphosis, as documented by the fossil record. *J. Morphol.* 156:53–126

38. Kukalova-Peck, J. 1983. Origin of the insect wing and wing articulation from the arthropodan leg. *Can. J. Zool.* 61:1618–69

39. LaFontaine, G. 1981. *Caddisflies*. New York: Winchester. 336 pp.

40. Lameere, A. 1917. Étude sur l'évolution des Éphémères. *Bull. Soc. Zool. Fr.* 42:41–59, 61–81

40a. Lehman, D., Kovav, N., eds. 1952. *Ephemera*. New York: Holt, Rinehart & Winston (Motion picture)

41. Levy, H. A. 1948. The male genitalia of Ephemerida (mayflies). *J. NY Entomol. Soc.* 56:25–41

42. Lyman, F. E. 1944. Effect of temperature on the emergence of mayfly imagoes from the subimago stage. *Entomol. News* 55:113–15

43. Maiorana, V. C. 1979. Why do adult insects not moult? *Biol. J. Linn. Soc.* 11:253–58

44. Marsh, N. 1983. *Trout Stream Insects of New Zealand.* Wellington, NZ: Millwood. 224 pp.

45. Matsuda, R. 1981. The origin of insect wings (Arthropoda: Insecta). *J. Insect Morphol. Embryol.* 10:387–98

46. McCafferty, W. P. 1979. Swarmfeeding by the damselfly *Hetaerina americana* (Odonata: Calopterygidae) on mayfly hatches. *Aquat. Insects* 1:149–51

47. McCafferty, W. P. 1981. *Aquatic Entomology.* Boston: Sci. Books Int. 448 pp.

48. McCafferty, W. P., Edmunds, G. F. Jr. 1979. The higher classification of the Ephemeroptera and its evolutionary basis. *Ann. Entomol. Soc. Am.* 72:5–12

49. McClure, R. G., Stewart, K. W. 1976. Life cycle and production of the mayfly *Choroterpes (Neochoroterpes) mexicanus* Allen (Ephemeroptera: Leptophlebiidae). *Ann. Entomol. Soc. Am.* 69:134–44

50. Morgan, A. H. 1911. May-flies of Fall Creek. *Ann. Entomol. Soc. Am.* 4:93–119

51. Morton, K. J. 1921. A new species of mayfly, *Palingenia* (sensu lato), from Mesopotamia. *Entomologist London* 54:177–80

52. Needham, J. G., Traver, J. R., Hsu, Y.-C. 1935. *The Biology of Mayflies,* Ithaca, NY: Comstock. 759 pp.

53. Penniket, J. G. 1962. Notes on New Zealand Ephemeroptera. II. A preliminary account of *Oniscigaster wakefieldi* McLachlan, recently rediscovered (Siphlonuridae). *Rec. Canterbury Mus.* 7:375–88

54. Percival, E., Whitehead, H. 1926. Observations on the biology of the mayfly, *Ephemera danica* Mull. *Proc. Leeds Philos. Lit. Soc. Sci. Sect.* 1:136–48

55. Pescador, M. L., Peters, W. L. 1980. A revision of the genus *Homoeoneuria* (Ephemeroptera: Oligoneuriidae). *Trans. Am. Entomol. Soc.* 106:357–93

56. Peters, W. L., Peters, J. G. 1977. Adult life and emergence of *Dolania americana* in northwestern Florida (Ephemeroptera: Behningiidae). *Int. Rev. Gesamten Hydrobiol.* 62:409–38

57. Pickles, A. 1931. On the metamorphosis of the alimentary canal in certain Ephemeroptera. *Trans. R. Entomol. Soc. London* 79:263–74

58. Poyarkoff, E. 1914. Essai d'une théorie de la nymphe des insectes holométaboles. *Arch. Zool. Exp. Gen.* 54:221–65

59. Rawlinson, R. 1939. Studies on the life-history and breeding of *Ecdyonurus venosus* (Ephemeroptera). *Proc. Zool. Soc. London Ser. B* 109:377–450

60. Riek, E. F. 1970. Ephemeroptera. In *The Insects of Australia,* ed. CSIRO, pp. 224–40. Carlton, Australia: Melbourne Univ. Press. 1029 pp.

61. Russev, B. K. 1973. Kompensationsflug bei der Ordnung Ephemeroptera. *Proc. 1st Int. Conf. Ephemeroptera, Tallahassee, Fla., (1970),* pp. 132–42. Leiden, The Netherlands: Brill

62. Schaefer, C. W. 1975. The mayfly subimago: a possible explanation. *Ann. Entomol. Soc. Am.* 68:183

63. Schoenemund, E. 1930. *Tierwelt Deutschland,* Teil 19, *Eintagsfliegen oder Ephemeroptera.* Jena, Germany: Fischer. 106 pp.

64. Sharov, A. G. 1966. *Basic Arthropodan Stock.* New York: Pergamon. 271 pp.

65. Snodgrass, R. E. 1954. Insect metamorphosis. *Smithson. Misc. Collect.* 122:1–124

66. Soldán, T. 1978. Revision of the genus *Palingenia* in Europe (Ephemeroptera, Palingeniidae). *Acta Entomol. Bohemoslov.* 75:272–84

67. Soldán, T. 1979. Spermatogenesis and oogenesis in mayflies (Ephemeroptera). *Proc. 2nd Int. Conf. Ephemeroptera, Krakow, Poland, (1975),* pp. 267–71. Warsaw/Krakow: Panstwowe Wydawnictwo Naukowe

68. Soldán, T. 1981. Secondary sexual characters in mayfly larvae and their evolutionary significance (Ephemeroptera). *Acta Entomol. Bohemoslov.* 78:140–42

69. Sternfeld, R. 1907. Die Verkümmerung der Mundteile und der Funktionswechsel des Darms bei Ephemeriden. *Zool. Jahrb. Anat.* 24:415–30

70. Stys, P., Soldán, T. 1980. Retention of tracheal gills in adult Ephemeroptera and other insects. *Acta Univ. Carol. Biol.* 1978:409–35

71. Swammerdam, J. 1675. *Ephemeri Vita.* Amsterdam: Wolfgang. 44 pp.

72. Swisher, D., Richards, C. 1971. *Selective Trout.* New York: Crown. 184 pp.

73. Taylor, R. L., Richards, A. G. 1963. The subimaginal cuticle of the mayfly *Callibaetis* sp. (Ephemeroptera). *Ann. Entomol. Soc. Am.* 56:418–26

74. Thorpe, W. H. 1950. Plastron respiration in aquatic insects. *Biol. Rev.* 25:344–90

75. Tiensuu, L. 1935. On the Ephemeroptera-fauna of Laatokan Karajala (Karelia Ladogensis). *Ann. Entomol. Fenn. Suom. Hyonteistiet. Aikak.* 1:3–23

76. Tillyard, R. J. 1926. *The Insects of Australia and New Zealand*. Sydney: Angus & Robertson. 560 pp.

77. Traver, J. R. 1932. Mayflies of North Carolina. *J. Elisha Mitchell Sci. Soc.* 47:85–236

78. Usinger, R. L. 1963. Introduction to aquatic entomology. In *Aquatic Insects of California*, ed. R. L. Usinger, pp. 3–49. Berkeley, Calif: Univ. Calif. Press. 508 pp.

79. Vogel, S. 1967. Flight in *Drosophila* III. Aerodynamic characteristics of fly wings and wing models. *J. Exp. Biol.* 46:431–43

80. Wesenberg-Lund, C. 1943. *Biologie der Süsswasserinsekten*. Berlin: Springer. 682 pp.

81. Wisely, B. 1965. Studies on Ephemeroptera III. *Coloburiscus humeralis* (Walker); morphology and anatomy of the winged stages. *NZ J. Sci.* 8:398–415