Flightlessness in mayflies and its relevance to hypotheses on the origin of insect flight

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Until now, only fully winged mayflies have been known. It has been proposed recently that brachyptery could be a missing link in the development of insect flight, via sailing or skimming aquatic insects. To our knowledge, we report here the first documented case of brachyptery in mayflies. The flightless genus *Cheirogenesia* is endemic to Madagascar, and the adults skim the water surface. This loss of the flight function has induced important physiological changes, such as a shift from lipids to carbohydrates in the energy reserves used during their adult life. Comparison of wing area of living mayflies with fossil species indicates that brachyptery could have already occurred in early flying insects (in the Permian). We argue that flight loss in *Cheirogenesia* has been made possible by the lack of fish predation in its natural habitats.

Keywords: Ephemeroptera; flightlessness; brachyptery; wings; evolution; energetics

1. INTRODUCTION

Mayflies (Ephemeroptera) play a key role in insect phylogeny even though their relationships with other insects are still under debate (Kristensen 1981, 1991; Riek & Kukalova-Peck 1984; Kukalova-Peck 1985).

They are the only pterygote insects that moult at the winged stage (subimago), and their origin goes back to the Carboniferous. Their ancestors were probably the first flying animals, and they are good candidates for the study of the evolution of wings and flight.

Insect wings are supposed to have evolved from mobile external gill plates (Kukalova-Peck 1978, 1983; Averof & Cohen 1997). Several hypotheses have been proposed to explain the evolution of wings, including courtship display, respiration, thermoregulation, aerodynamic functions (Kingsolver & Koehl 1994). Recently, much debate has been provoked by Marden & Kramer whose studies were based on brachypterous (i.e. with reduced wing length) stoneflies (Plecoptera). They proposed new interpretations of the origin of insect flight, namely skimming the water surface (Marden & Kramer 1994) and/or sailing across it (Marden & Kramer 1995). The authors claim that these forms of locomotion are plesiomorphic traits that could represent intermediate stages in the evolution of flight. If so, we could better understand why wings that are not fully developed have still been preserved by natural selection. Brachyptery in this case could be a missing link leading to macropterous (i.e. with fully developed wings) insects.

Flightlessness among insects is a phenomenon that occurs in nearly all winged orders of insects (Wagner & Liebherr 1992; Roff 1994). Up until now, mayflies were supposed to be always macropterous in both sexes (Hubbard 1979), although strong reduction of hindwings or even complete absence of them may be observed in some independent lineages such as Baetoidea, Leptophlebioidea and Caenoidea.

The genus *Cheirogenesia* (Palingeniidae) is endemic to Madagascar. Supposed to be monospecific until recently, it is composed of several geographic vicariant species as the result of strong microendemism among river basins (Sartori & Elouard 1999). This genus forms an ancient lineage and is the sister group to all other Palingeniidae.

The main difference between *Cheirogenesia* and other palingeniid species, as well as between other mayflies in general, is that *Cheirogenesia* winged stages are unable to fly. Instead, they skim the water surface where searching for mates, mating and oviposition take place. Wing movements propel them at high speed, with only their legs being in contact with the water (as in the manner of hydrofoil ships). In this paper, we present results of a three-year study on this peculiar genus. To our knowledge, brachyptery and flightlessness are reported for the first time in the order Ephemeroptera, and a first attempt is made to consider Marden & Kramer's hypothesis in the light of data obtained from extant and fossil mayflies.

2. MATERIAL AND METHODS

Thirty-two extant mayfly species were chosen for morphometric analysis. These species cover the major phylogenetic lineages of the order as presently proposed (McCafferty 1991). Ten specimens were used for each species, except for those from the literature for which standard deviation is missing (table 1). Computation of wing areas was determined either from the available literature or from material kept in the collections of the Museum of Zoology, Lausanne. Right fore- and hindwings were detached, drawn with the help of a camera lucida, scanned, and wing area computed using standard drawing software such as Canvas.

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Table 1. *Number of specimens, origin and references of the species used for plotting the data of figure 1*

(Systematic arrangement according to Hubbard (1987, 1990). References as follows: 1, collections of the Museum of Zoology, Lausanne; 2, Gillies (1960); 3, Demoulin (1954); 4, Demoulin (1956); 5, Demoulin (1955); 6, Demoulin (1965); 7, Carpenter (1992); 8, Kukalova-Peck (1985).)

species	males	females	country	reference
Baetoidea				
Siphlonuridae				
Siphlonurus aestivalis	5	5	Switzerland	1
Baetidae				
Baetis fuscatus	5	5	Switzerland	1
Centroptilum dicentrum	1	—	Zaïre	4
Cloeon dipterum	5	5	Switzerland	1
Heptagenioidea				
Heptageniidae				
Ecdyonurus helveticus	5	5	Switzerland	1
Electrogena lateralis	5	5	Switzerland	1
Epeorus sylvicola	5	5	Switzerland	1
Rhithrogena iridina	5	5	Switzerland	1
Stenonema mexicanum	2	5	USA	1
Oligoneuriidae				
Oligoneuriopsis orontensis	5	5	Israel	1
Ephemerelloidea				
Ephemerellidae				
Ephemerella ignita	5	5	Switzerland	1
Tricforythidae				
Ephemerythus niger	1	—	Tanzania	2
Leptophlebioidea				
Leptophlebiidae				
Fulleta dentata	1	—	Zaïre	3
Fulletomimus marlieri	1	—	Zaïre	4
Habroleptoides confusa	5	5	Switzerland	1
Nathanella indica	1	—	India	5
Caenoidea				
Caenidae				
Caenis lactea	5	4	Switzerland	1
Neoephemeridae				
Neoephemera youngi	—	5	USA	1
Ephemeroidea				
Polymitarcyidae				
Ephoron virgo	5	5	France	1
Tortopus incertus	5	5	USA	1
Behningiidae				
Dolania americana	5	5	USA	1
Ephemeridae				
Eatonica schoutedeni	—	1	Ivory Coast	1
Ephemera danica	5	5	Switzerland	1
Hexagenia limbata	—	2	USA	1
Proboscidoplocia leplattenierae	2	4	Madagascar	1
Palingeniidae				
Anagenesia tenera	1	1	Java	6
Cheirogenesia decaryi	5	5	Madagascar	1
Cheirogenesia edmundsi	5	5	Madagascar	1
Cheirogenesia laurencae	5	5	Madagascar	1
Palingenia longicauda	5	3	Hungary	1
Plethogenesia bibisica	1	—	New Guinea	6
Plethogenesia papuana	—	1	New Guinea	6
Protereismatoidea[a]				
Protereismatidae				
Protereisma permianum	1	—	USA (Permian)	7
Misthodotidae				
Misthodotes delicatulus	1	—	USA (Permian)	8

[a]Two fossil species included from this superfamily.
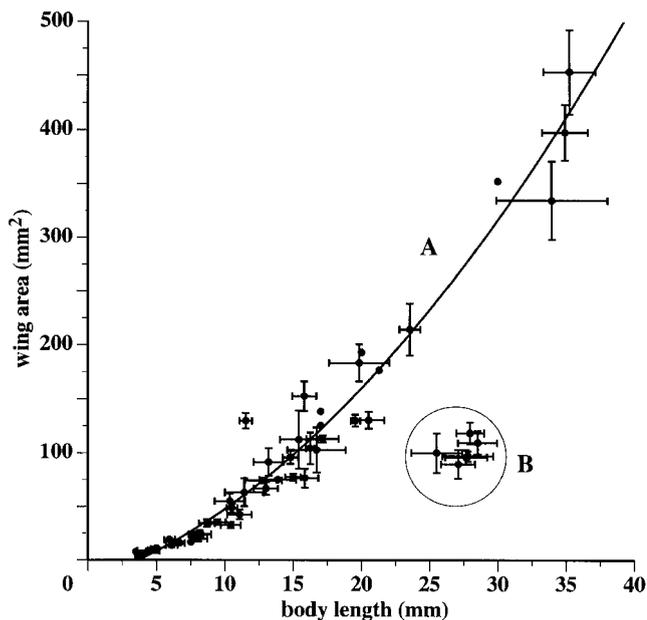
Figure 1. Relationship between body length (mm) and wing area of extant species (fore- and hindwings) (mm²) with standard deviation. (*a*) Values for the flying species; (*b*) values for *Cheirogenesia* spp. Analysis is based on 54 plotted values considering that sexes are analysed independently (see table 1). The two fossils are excluded for size reasons.

Two fossil species were added to the data set, *Protereisma permianum* (Carpenter 1992) and *Misthodotes delicatulus* (Kukalova-Peck 1985). They belong to the superfamily Protereismatoidea, which includes the oldest and closest relatives to modern species (Hubbard 1987). In the available literature, these two species were among the few suitable for our purpose, i.e. wings well preserved and body length known. In order to allow comparison, a ratio between square-root transformed wing area and body length was calculated. Kruskal–Wallis tests and *a posteriori* multiple comparisons between treatments were used.

To investigate if the loss of flight results in physiological changes, mature nymphs and exhausted adults of *Cheirogenesia edmundsi* were caught at the Mangoro River (Madagascar) in May 1995. Each individual was cut to remove the abdomen (especially eggs) in order to analyse the biochemical components only in the head and thorax. Lipid and glycogen content was determined according to Van Handel (1985*a,b*) and Ruffieux (1997). Mann–Whitney *U*-tests were used to compare the results.

3. RESULTS

Figure 1 presents the morphometric results. Excluding *Cheirogenesia* spp. (figure 1*a*), there is a clear correlation between body length and wing area. This relationship can be expressed by a second-degree polynomial equation: $y = 0.22x^2 + 4.55x - 19.97$. *Cheirogenesia* specimens (figure 1*b*) are markedly distinguished from the model by having comparatively smaller wing area. With respect to volant mayflies, *Cheirogenesia* species show a reduction of wing length of *ca.* 20%, and above all a reduction of wing area of *ca.* 50%, i.e. they should normally have a wing area two times greater than that exhibited.

The ratio between square-root transformed wing area and body length averages 0.642 (± 0.078 s.d.) for extant mayfly species, 0.446 (± 0.022 s.d.) for the two fossil

records, and 0.368 (± 0.019 s.d.) for *Cheirogenesia* species. A Kruskal–Wallis test indicates significant differences among the three samples ($H = 20.20$, $p < 0.001$). *A posteriori* multiple comparisons indicate that differences were significant between *Cheirogenesia* and other extant mayflies ($Z = 4.11$, $p < 0.001$), but not between fossils and *Cheirogenesia* ($Z = 2.12$, $p = 0.20$) or between fossils and extant mayflies ($Z = 0.30$, $p > 0.99$).

Table 2 summarizes the results obtained with the lipid and glycogen content of *Ch. edmundsi*. No significant decrease in lipid content was observed during adult activity, for both males and females, which suggests that this component does not play a significant role during locomotion. However, a strong decline in glycogen content was found in both sexes.

4. DISCUSSION

Cheirogenesia winged stages do not perform flight but skim the water surface. Male imagoes, when tossed into the air, are unable to sustain their body weight and so fall. As this flightlessness is the consequence of a pronounced decrease in wing surface rather than wing length reduction, we call it cryptic brachyptery. This phenomenon is reported for the first time in mayflies and should be taken into account when (re)studying fossil data. Our results show that, despite the lack of significant differences, the two fossil species analysed have a ratio intermediate between *Cheirogenesia* and other extant mayflies, suggesting that brachyptery could have occurred in Palaeozoic winged forms (Permian mayflies, *ca.* 260 million years (Ma) before present (BP)). Flightlessness in *Cheirogenesia* has induced other important morphological changes, especially in the structure and shape of the forewing (figures 2 and 3). The wing surface and margin are covered with water-resistant scales (figure 3) that provide them with hydrofuge properties; homologous structures are present in subimagoes, but quite uncommon in mayfly adults (Edmunds & McCafferty 1988). A unique feature among extant mayflies can be found in the wing articulation, where the platform and arch are desclerotized, creating a deformable zone that allows larger amplitude strokes and moving of the wings in the horizontal plane, a necessary condition for skimming (figure 2*b*). This pattern of wing articulation is thought to have existed in the first winged insects (Brodsky 1994). The bat-like wing is the reason for the generic name *Cheirogenesia*. But analogies may go further (Vaughan 1970). The trailing edge of the *Cheirogenesia* forewing has considerable give when subjected to air pressure, compared to the rigid posterior margin of volant mayflies (figure 2*a,b*). Moreover, skimming locomotion requires thrust rather than lift, which is produced mainly by the distal half of the wing.

An interesting problem is to establish whether this peculiar locomotion has physiological consequences. In all mayflies already investigated (Sartori *et al.* 1992; Ruffieux 1997), glycogen content is always lower than 2 joules per individual and lipids are the main fuel consumed during flight. As these insects have to sustain long flights (e.g. males waiting for females, females flying upstream for oviposition), lipids are the most appropriate fuel to be used. In *Cheirogenesia*, no significant decrease in

Table 2. *Analyses of the energetic components in the head and thorax of* Cheirogenesia edmundsi *specimens*

(Content is expressed in joules±standard deviation. n, number of specimens analysed; p, significance in Mann–Whitney U-test.)

| stage	lipid content (joules) value±s.d.	n	p	glycogen content (joules) value±s.d.	n	p
male nymphs	45.905±3.227	13		6.269±0.603	10	
			n.s.			<0.001
male imagoes	40.285±2.210	19		2.745±0.318	10	
female nymphs	31.216±2.540	8		5.005±0.410	10	
			n.s.			<0.001
female imagoes	28.923±1.063	25		1.302±0.062	15	
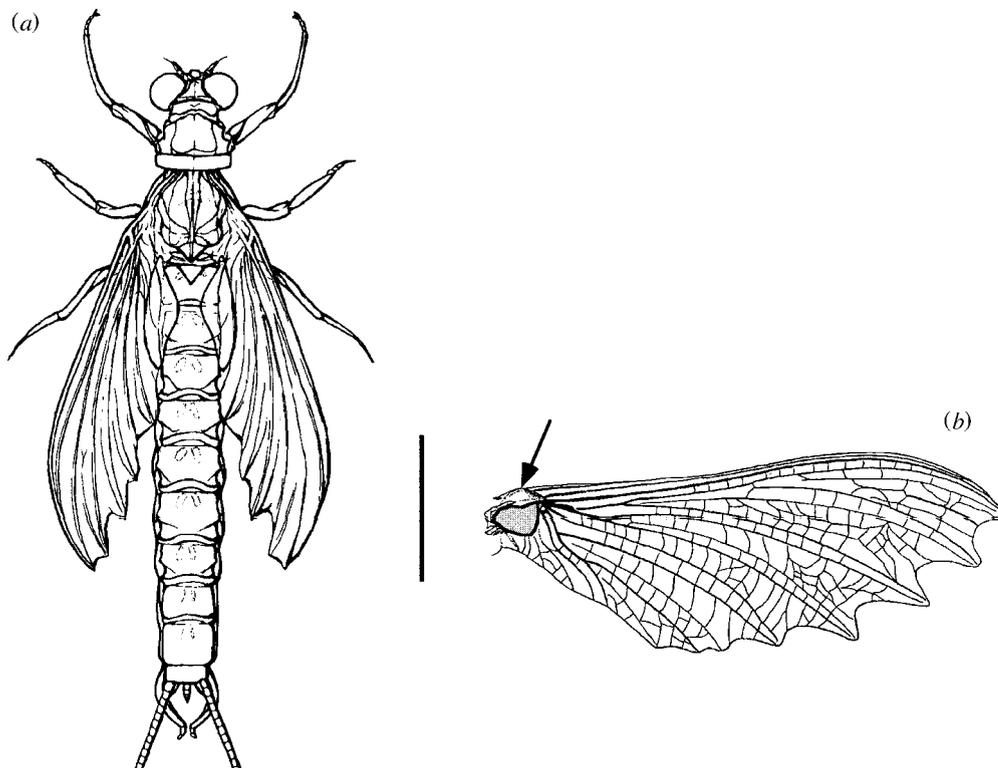
Figure 2. Male habitus of the flightless mayfly *Cheirogenesia decaryi* (*a*), and detail of the forewing (*b*), showing the very special bat-like posterior margin, as well as the highly specialized gemination of longitudinal veins. Desclerotized arch (arrow) and platform (in grey) are indicated. The shape of the posterior margin of the forewing may also be a kinematic adaptation for skimming rather than flying (see text). Scale bar, 5 mm.
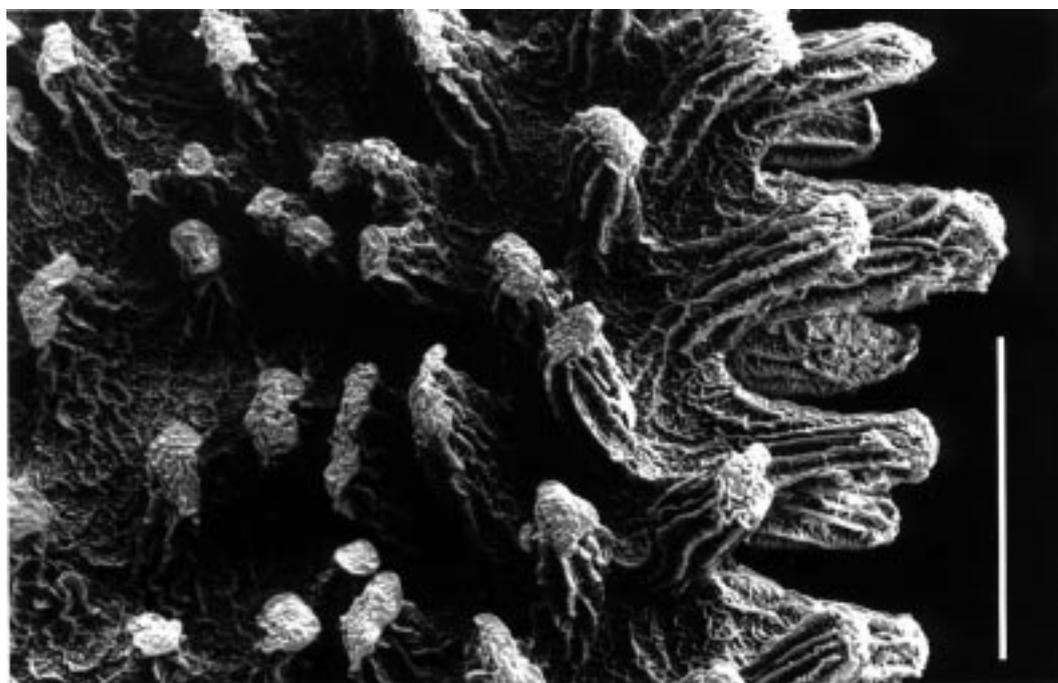
Figure 3. Ultra-structure of the wing surface and margin reveals unique features never found in any other mayfly species. The presence of these upright scales certainly plays a hydrofugous role and enables the wing to remain waterproof. Scale bar, 10 μm.

lipid content has been noticed during adult life span, suggesting that skimming behaviour does not require this type of energy reserve. Moreover, glycogen content is twice to three times more important in *Cheirogenesia* than in flying species. Even though glycogen produces less energy than lipids (w/w), the only significant decrease ($p < 0.001$) in energy reserves affects the glycogen content, which represents the only rapidly available energy source, and could be sufficient to skim the water surface. This skimming behaviour needs less energy than flight, resulting in a different energy strategy, which should allow allocation of more energy (lipids) to reproduction, or lead to a shorter larval development in comparison with flying species.

An important question is whether the peculiar features observed, particularly brachyptery, are primitive or derived characters. In our opinion, there is no doubt that brachyptery in extant mayflies appeared secondarily. Wing venation of *Cheirogenesia* presents the same structure as in other flying palingeniid mayflies. In particular, gemination of longitudinal veins (figure 2*b*), i.e. the subparallel arrangement of longitudinal and intercalary veins, is a highly specialized trait that has appeared independently in several lineages such as Oligoneuriidae and Benhingiidae. No ancient fossil records present such peculiar venation, so gemination of the longitudinal veins can be considered as a derived character. Reduction of wings, apart from our present observations on the Proter-eismatoidea, has never been postulated in fossil mayflies. Brachyptery is certainly an autapomorphy shared by *Cheirogenesia*, as proposed for modern stoneflies (Will 1995; Thomas & Norberg 1996).

So, if brachyptery and flightlessness in mayflies are derived characters, why give up flight? Among insects, flightlessness increases with altitude and latitude, or may be correlated with reduction of plant production at lower temperatures (Roff 1990). These constraints do not seem to apply to *Cheirogenesia*, which lives in subtropical environments.

One of the main constraints on aquatic insects such as mayflies and stoneflies is to escape the water surface, to avoid predation mainly by insectivorous fishes. Observations on another palingeniid species (*Palingenia longicauda*) show that, during the emergence period, fish predation is extremely important (M. Sartori and L. Ruffieux, unpublished data). Thus, we could assume that if selective pressures such as fish predation are missing, natural selection would favour less energy demanding locomotion on the water surface.

It has been shown (Kiener 1963; Stiassny & Raminosoa 1994), that the freshwater fish fauna in Madagascar is incredibly poor (62 species) owing to isolation of the island from Africa *ca.* 120 Ma BP. Malagasy rivers of the Central Area where most of the *Cheirogenesia* populations are located are entirely lacking in indigenous insectivorous fishes (de Rham 1996).

Although flight loss by release from predators is well documented in island birds (Livezey 1993; McNab 1994), no evidence has ever been proposed that such a mechanism could also be applied to insects. Our hypothesis is that the lack of selective pressures caused by the absence of predatory fishes has induced the loss of flight and the reduction of the wing surface leading to

brachyptery. In the investigated rivers, the most abundant aquatic predators were whirligig beetles (Gyrinidae). The activity period of *Cheirogenesia* adults occurs at night (from *ca.* 04.45 until dawn), when beetles are normally not active.

This constraint being resolved, there remains the problem of dispersal. In environments such as those colonized by *Cheirogenesia* populations (large meandering rivers with highly homogeneous substratum), dispersal is less vital and does not necessarily require flight abilities, contrary to the case of other species living in much more heterogeneous habitats such as brooks or streams. It has been often reported that the loss of flight in insects is more effective in stable environments in which long-term persistence is unlikely to require long-distance dispersal (Roff 1990). Moreover, flightlessness allows females to allocate more resources to egg production (Roff 1994). Together with the shift from lipids to glycogen for locomotion, *Cheirogenesia* females are able to increase their reproductive success.

Mayflies are among the first winged insects that appeared on earth, at a time when conditions, such as stable environment and absence of fish predation, were probably analogous to those observed actually in Madagascar. Development of wings to perform flight may have occurred when these conditions changed. Thomas (1996) also supported the idea that insect flight evolved in response to a constant selection pressure for increased locomotor performance. Our data suggest that in some Palaeozoic mayflies, plesiomorphic brachyptery may have occurred and could be a missing link leading to macroptery. We hypothesize that flightlessness (via brachyptery) in extant mayflies constitutes an exceptional secondary trait found in long-isolated populations and is to be explained by the removal of predation in permanent and homogeneous habitats. Although skimming behaviour in mayflies and stoneflies represents a homoplasy, the (re)study of existing fossil records in the light of what we call cryptic brachyptery could bring new evidence and support to Marden & Kramer's hypothesis on the origin of insect flight.

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