

Rowing locomotion by a stonefly that possesses the ancestral pterygote condition of co-occurring wings and abdominal gills

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Received 17 July 2002; accepted for publication 14 November 2002

A leading hypothesis for the origin of insect wings is that they evolved from thoracic gills that were serial homologues of the abdominal gills present in fossil pterygotes and in the nymphs of some modern mayflies, damselflies and stoneflies. Co-occurrence of thoracic wings and abdominal gills is the primitive condition for fossil pterygote insects, whereas the winged stage of modern insects almost exclusively lacks abdominal gills. Here we examine the locomotor behaviour and gill morphology of a stonefly, *Diamphiphnopsis samali* (Plecoptera), which retains abdominal gills in the winged adult stage. This species can fly, but also uses its forewings as oars to accomplish rowing locomotion along the surface of water. The abdominal gills are in contact with both air and water during rowing, and their elaborately folded surface suggests an ability to contribute to gas-exchange. *D. samali* nymphs also have behaviours that place them in locations where their gills are exposed to air; they forage at night at the stream margin and within bubble curtains in rapids. These traits may exemplify an early pterygote condition in which gill and protowing function overlapped in an amphibious setting during a transition from aquatic to aerial locomotion and gas exchange. Rowing locomotion provides a novel and mechanically intermediate stage for the wings-from-gills and surface-skimming hypotheses for the origin of insect wings and flight. © 2003 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2003, 79, 341–349.

ADDITIONAL KEYWORDS: amphibious – Chile – *Diamphiphnopsis* – evolution – flight – insect – Plecoptera – surface skimming.

INTRODUCTION

‘Adult tracheal gills are probably “living fossils” among the organs of modern adult pterygotes. Their development apparently was so deeply rooted in the epigenotypes of the ancestors of modern perennibranchiate [i.e. gilled adult] species that it has not been entirely suppressed by metamorphosis and long non-functionality during the more than 300 million years of the terrestrial existence of adult pterygotes. Hence, perennibranchiality is not merely an interesting aberrant phenomenon, but a remnant of a memorable and still largely unknown past of insects, and probably also a partial key to it.’ Štys & Soldán (1980: 432–433).

The evolution of flight in insects appears to have triggered an unparalleled radiation and diversification, and today the pterygote insects comprise over half of all described species (Hammond, 1992). However, the fossil record contains none of the early transitional stages leading to or between apterygote and pterygote insects. As a result, the evolutionary origin of insect flight remains uncertain and is a popular topic for study and lively debate by biologists and palaeontologists (e.g. Kukalova-Peck, 1978; Kingsolver & Koehl, 1985; Ellington, 1991; Marden & Kramer, 1994; Dudley, 2000).

Key issues in these debates are the phylogenetic origin of insects and the anatomical origin of wings. One prominent hypothesis is that insects are a sister clade of myriapods, whose wings arose from lateral outgrowths of the dorsal thorax. This hypothesis envisions flight originating in a terrestrial environment, most probably by a sequence involving parachuting,

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gliding, and ultimately flapping, during which the immobile lateral outgrowths of the thorax (proto-wings) gradually acquired the articulation, musculature and neural pattern necessary for powered flight. This hypothesis has traditionally maintained a prominent place in all debates and is favoured by many contemporary authors (e.g. Grodnitsky, 1999; Dudley, 2000).

A competing hypothesis is that insects are a sister clade of crustaceans, whose wings evolved from mobile gills that already had the articulation, musculature and neural pattern required for creating fluid dynamic forces. This hypothesis has received support from recent phylogenetic and developmental studies (see below), yet it remains to be determined precisely how gills could have evolved into wings. In the study presented here, our aim is not to resolve competing phylogenetic and anatomical hypotheses, but rather to use the wings-from-gills model as a starting point to examine the behaviour and morphology of one modern species, *Diamphipnopsis samali* Illies (Plecoptera, Eusthenioidea, Diamphipnoidae), that may have particular bearing on how gills could have evolved into wings.

Recent phylogenetic analyses based on molecular (Friedrich & Tautz, 1995; Regier & Schultz, 1997; Aguinaldo & Lake, 1998; Boore, Lavrov & Brown, 1998; Winnepeninckx, Van de Peer & Backeljau, 1998; Garcia-Machado *et al.*, 1999; Hwang *et al.*, 2001), morphological (Strausfeld, 1998; Strausfeld *et al.*, 1998) and combined data sets (Giribet, Edgecombe, & Wheeler, 2001) support the hypothesis that insects are a sister clade of the Crustacea (see also *Annales de la Société Entomologique de France (N.S.)*, 2001, 37(1–2) for a mixture of supporting and conflicting viewpoints). Recent data also indicate a homology between crustacean gills and insect wings, based on the demonstration that two of the genes involved in morphogenesis of a dorsal exite of the brine shrimp gill are involved in a similar stage of development in *Drosophila* wings (Averof & Cohen, 1997). *Drosophila* carrying mutant forms of *ultra-bithorax* and *abdominal-a* homeotic genes develop appendage primordia on their abdominal segments (Carroll, Weatherbee & Langeland, 1995) in the same locations that both fossil and certain modern insect nymphs have gills. Based on fossil evidence, these gills have long been argued to be serial homologues of the thoracic appendages that became wings (Kukalova-Peck, 1978, 1983, 1987, 1991). Thus, it appears that modern homeotic regulation represses the initiation of wing/gill development on all but the meso- and metathorax of extant adult pterygotes (Averof & Akam, 1995; Carroll *et al.*, 1995; Galant & Carroll, 2002; Ronshaugen, McGinnis & McGinnis, 2002; but see Jockusch & Nagy, 1997 and Williams, 1999 for notes of

caution regarding this interpretation). In addition, the wings of modern insects carry sensilla and chemoreceptors that are present on arthropod appendages (such as the leg exites that are hypothesized to have become gills) but not on the thoracic body wall (Dickinson, Hannaford & Palka, 1997). Together, these studies support the hypothesis that insects and their wings evolved from crustacean-like ancestors with moveable leg-derived gills.

Additional support for the wings-from-gills model comes from fossils of early pterygotes. A number of early fossil insects possess both thoracic wings and gill-like structures on their abdomen; these fossils have led Kukalova-Peck (1991) to conclude that the coexistence of wings and abdominal plate gills is the primitive pterygote condition. Fossil insects possessing both wings and what appear to be gills, from the upper Carboniferous and lower Permian, are abundant and taxonomically diverse (see Fig. 1a–d; Kukalova-Peck, 1978, 1991), including examples from the stem groups that led to modern Ephemeroptera, Hemiptera and Plecoptera, along with the extinct order Megasecoptera. Although it cannot be determined with certainty that these structures did in fact serve as gills, or even if these insects were associated with water, the similarity of the structures in Figure 1a–c with gills of modern mayflies, Euphaeid damselflies and Eusthenioid stoneflies makes this a reasonable interpretation.

The foregoing evidence is part of the empirical basis for the wings-from-gills model, but for this hypothesis to be robust it also requires a plausible mechanical pathway that could have transformed the structure and function of appendages that served originally as gills to appendages that served ultimately as wings. In this regard, the behaviour and mechanics of modern Plecoptera and Ephemeroptera (stoneflies and mayflies; these two orders arose near the base of the early split between Neoptera and Palaeoptera and are thought to have retained many primitive morphological features) are potentially informative. Various stoneflies and mayflies use aerodynamic locomotion to move in two dimensions across the surface of water, in some cases by using fairly rudimentary wings and wing motions (Marden & Kramer, 1994, 1995; Kramer & Marden, 1997; Ruffieux, Elouard & Sartori, 1998; Marden *et al.*, 2000). In stoneflies, these behaviours are basal and phylogenetically diverse (Thomas *et al.*, 2000). Here we extend that line of studies by examining *D. samali* stoneflies that possess the primitive pterygote condition of co-occurring wings and abdominal gills.

Traditionally, a fundamental difficulty for the wings-from-gills hypothesis was the assumption that terrestriality and tracheal gas exchange are primitive traits for insects, dating back at least as far as a ter-

restrial Hexapoda–Myriapoda stem group (Pritchard *et al.*, 1993). However, the emerging picture of a crustacean-like root for insects makes it much more plausible that tracheal gas exchange was an evolutionary novelty in early insects, and that apterygote insects lost gills (perhaps independently, since apterygotes do not appear to be a monophyletic group) as they invaded land, thereby diverging from an aquatic or semi-aquatic gill-bearing stem (Toms, 1984) that gave rise ultimately to pterygote insects. Trachea may have evolved as simple invaginations that enhanced aerial gas exchange in an amphibious setting, then subsequently became elaborated so that they extended into pre-existing structures such as gills (Štys & Soldán, 1980). This scenario suggests that tracheal gas exchange evolved gradually in early insects, overlapping with and ultimately replacing an ancestral blood-based gas exchange system that presumably used haemocyanin, the predominant oxygen-binding pigment in the blood of non-tracheate arthropods.

At least one species of modern insect (an orthopteran) possesses a haemocyanin gene and expresses a protein that appears to be capable of encoding a functional oxygen transport molecule (Sanchez *et al.*, 1998). Consistent with the molecular phylogenetic evidence discussed above, this gene clusters with crustacean haemocyanin in a molecular phylogenetic analysis (Burmester, 2001). Its presence in a modern insect suggests that there could have been a partial reliance on blood-based gas exchange in early pterygotes, and there may even persist some remnants of this ability in certain modern species. There are no known examples of insects that use haemocyanin for gas exchange, but it appears that the relatively basal orders of insects (as opposed to more derived Diptera, Lepidoptera and Hymenoptera) have not been examined for this ability. We reasoned that an insect that has gills in the adult stage might also possess the ancestral trait of partial reliance on blood-based gas exchange. Thus, we tested *D. samali* haemolymph for the ability to reversibly bind oxygen.

METHODS

We examined *Diamphipnopsis samali* stoneflies in southern Chile during January 2000. Nymphs and adults were collected from the Estero Anihueraqui (39°25' S, 71°33' W), a steep, fast-flowing, oligotrophic and minimally human-impacted stream that drains mountains along the border between Chile and Argentina. The study site is accessible from a secondary road that runs parallel to the east side of the Rio Trancura, south of the town of Curarrehue.

Adults were attracted at night to a black-light. Nymphs were collected from under rocks along the stream bottom during the day, or while foraging in the

open at night. After capture, stoneflies were maintained in an insulated cooler at approximately 10–15°C.

We observed flight ability by releasing freshly captured adults (1–3 h post-capture) indoors and making a qualitative characterization according to criteria described in Marden *et al.* (2000). Surface-skimming behaviours were examined by dropping adults onto the surface of a shallow tank of water (40 × 50 × 4 cm), where they were filmed at 500 frames per second with a high-speed video recorder (Redlake HR 1000). Velocities were measured from the subset of records in which the insect's movement was nearly parallel to the film plane. Air and water temperature for these experiments was 18–22°C.

Haemolymph was collected from nymphs and adults, centrifuged at 3000 G for 5 min to remove cells, and then frozen. These samples were kept frozen on dry ice during transportation to our laboratory at Pennsylvania State University, where they were stored at –80°C. To determine if *D. samali* haemolymph is capable of reversibly binding oxygen, we diluted 50 µL samples of haemolymph in 800 µL of a pH 7.0 solution that matched the ionic concentration of Plecoptera haemolymph (Sutcliffe, 1962). As a positive control, we collected haemolymph from a blue crab (*Callinectes sapidus*), centrifuged as above to remove cells, and stored at –80°C. Crab haemolymph was diluted in a crustacean ringers solution at pH 7.4. Diluted samples of haemolymph were thawed and placed in a cuvette through which we bubbled known mixtures of nitrogen and oxygen gas. By varying the concentration of oxygen between 0 and 21%, and determining changes in light absorbance at a wavelength of 340 nm, we were able to obtain oxygen-binding profiles for haemocyanin (e.g. Innes & Taylor, 1986).

RESULTS

We captured six adult *D. samali* stoneflies at our black-light, one of which was observed flying as it approached the light. The remaining individuals either arrived at the light without our notice (unlikely given their 25 mm body length and 70 mm wingspan) or crawled to it from surrounding vegetation. Thus, we made only minimal observations of their flight behaviour in the field. All six *D. samali* adults were readily stimulated to fly indoors. These flights lasted only a few seconds, during which they showed very gradual climb rates and little or no ability to manoeuvre or avoid stationary objects. Despite a small sample of observations, this species clearly lacks the vertical acceleration and manoeuvrability of stoneflies that we have previously classified as strong fliers. Rather, they are marginal fliers according to the three-tiered qualitative scheme (strong, marginal or flightless) that we

have used to describe stonefly flight ability (Marden *et al.*, 2000).

When placed on a water surface, all of the *D. samali* adults used a novel and stereotypical combination of drag- and lift-based mechanisms to accomplish rowing locomotion (see <http://www.bio.psu.edu/people/faculty/marden/movies/rowing.mov>). The following data were obtained from two video recordings in which the position of the insect provided excellent visual resolution of the wing kinematics and the forward velocity of the body. The forewings were elevated to only about 45–60° above the horizontal at the dorsal apex of the wingstroke (Fig. 1h). At the bottom of the downstroke (Fig. 1j), the forewings made broad and prolonged contact with the water. During the interval between the end of the downstroke and the start of the upstroke (Fig. 1j,k), the distal 25–50% of the ventral forewing surface laid flat upon the water while the wingtip moved posteriorly before the initiation of the upstroke

(Fig. 1j,k). Rearward movement of the forewing while it was in contact with the water created a drag-based, rowing stroke that propelled the insect forward. The forewing kinematics featured a long duty cycle for the rowing portion of the stroke, in which time on the water exceeded time in the air by a factor of 1.9. The hindwing kinematics indicated a more typical lift-based function, with a 90° stroke angle, a peel (Ellington, 1984) at the start of the downstroke, and relatively little contact with the water.

Despite its large size, *D. samali* had an unusually low forward velocity (mean 23 cm s⁻¹, s.d. 1.5) during rowing (Fig. 2; range 15–63 cm s⁻¹ in 17 species from seven families; data from Marden *et al.*, 2000). Wingbeat frequency (17 Hz, s.d. 1.1) was also among the slowest that have been recorded from surface skimming stoneflies (20–45 Hz; Marden *et al.*, 2000), although this is consistent with the expected inverse scaling of frequency and wing length.

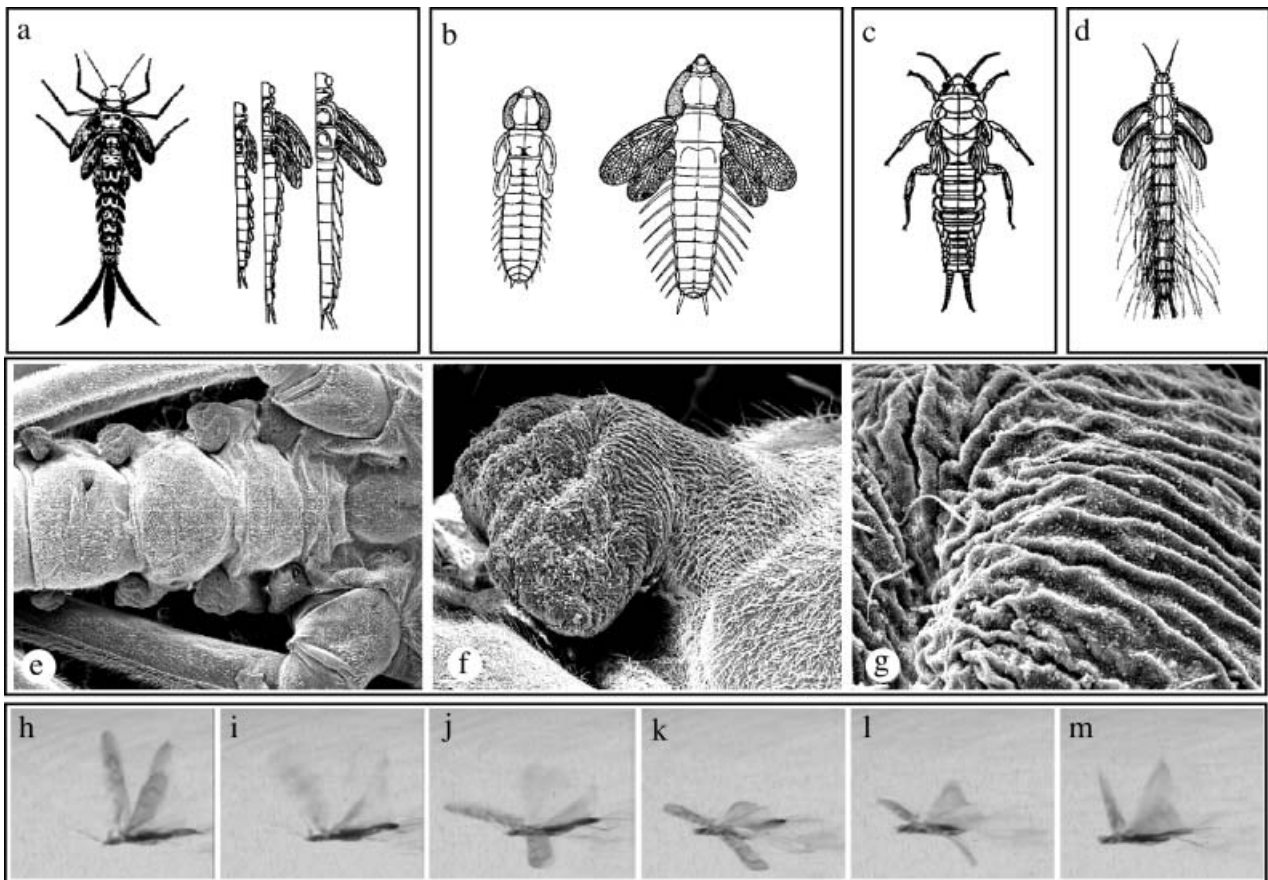


Figure 1. (a–d) Drawings (from Kukalova-Peck, 1978, 1991) of fossil representatives of the stem groups that led to the modern orders (a) Ephemeroptera, (b) Hemiptera, (c) Plecoptera, and (d) a representative of the extinct order Megaseoptera. (e–g) Scanning electron micrographs of the gills of *D. samali* adults on abdominal segments 1–4. A close-up image (g) of a single gill (f) reveals extensive folding that enhances surface area, a characteristic of functional exchange surfaces. (h–m) Single frames from high speed video (see <http://www.bio.psu.edu/People/Faculty/Marden/movies/rowing.mov>) of rowing locomotion. The forewings are in contact with the water surface in images (j) and (k).

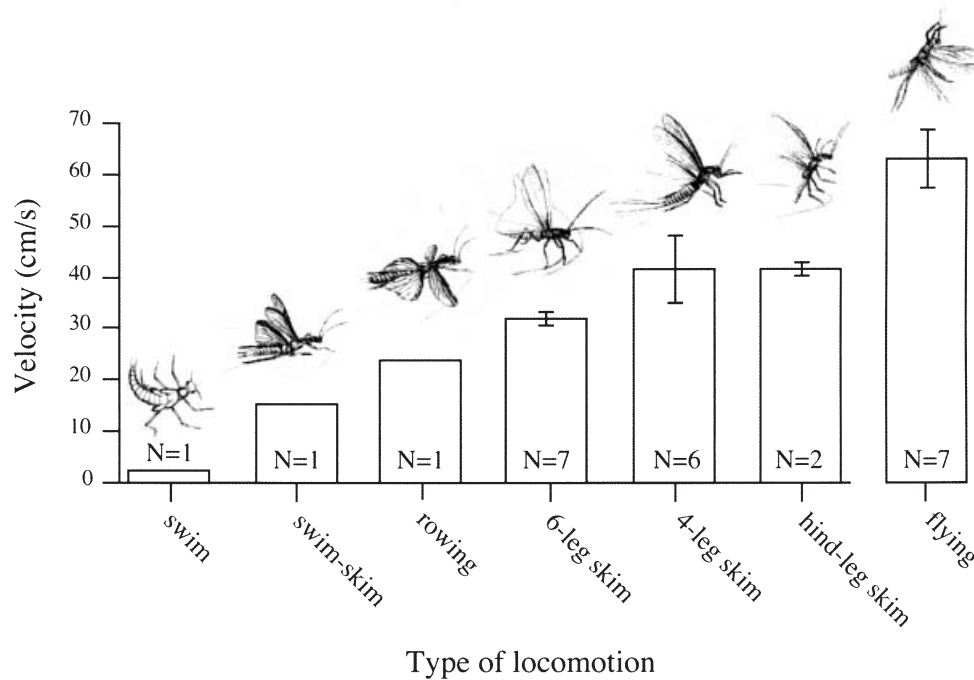


Figure 2. Mean velocity of stoneflies using different forms of aquatic, semi-aquatic, and aerial locomotion. Sample sizes refer to the number of species; error bars represent the standard error of the grand mean (i.e. variability among means derived from multiple species). For behaviours other than rowing, data are from Marden *et al.* (2000).

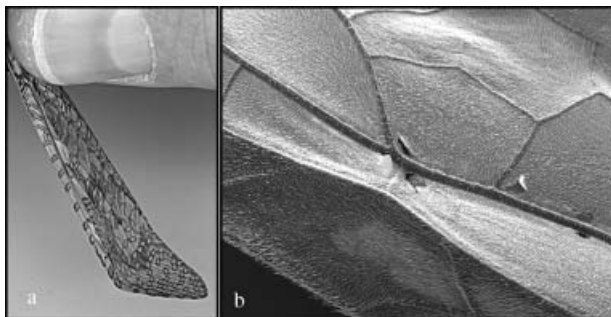


Figure 3. (a) A forewing of *D. samali* held with the tip on a water surface to illustrate the flexion line that enhances rowing function. (b) Bends in the radial and radial sector veins that create the flexion line.

The forewing of *D. samali* has a line of flexion that allows the distal portion to bend (Fig. 3a) and lay flat upon the water during the rowing portion of the stroke, and to be smoothly removed from the water surface at the start of the upstroke. The flexion line is created by termination of the subcostal vein at a point that is just proximal to where the radial and radial sector veins bend toward each other (Fig. 3b; vein terminology according to Borror, DeLong & Triplehorn, 1981). This vein arrangement creates a region where the stiffness of the wing is much reduced

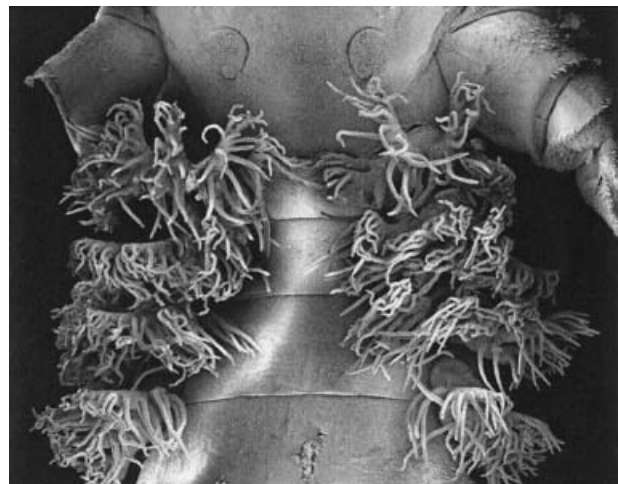


Figure 4. Scanning electron micrograph of the elaborately branched gills on the first four abdominal segments of a *D. samali* nymph.

compared to more proximal regions. This type of wing flexion is common in Plecoptera, Megaloptera and Mecoptera (Wootton, 1981; Brodsky & Ivanov, 1983).

Diamphipnopsis samali nymphs have gills on abdominal segments 1–4. These gills are articulated at their base, elaborately branched (Fig. 4), and are

flapped during aquatic gas exchange (see <http://www.bio.psu.edu/People/Faculty/Marden/movies/gillflap.mov>; note that this disproves the assertion that eustheniid and diamphipnoid stoneflies have little or no gill movement (Zwick, 2000)). *Diamphipnopsis samali* adults also have gills on abdominal segments 1–4 (Fig. 1e–g). Adult gills lack the articulation and elaborate branching of the nymphal gills, but extensive folding (Fig. 1g) enhances surface area and hints that these gills retain some degree of function in gas exchange. Adult gills also lack wet-resistant hairs that are ubiquitous on adjacent body regions and on the wing surfaces. Unlike most other stoneflies (Marden *et al.*, 2000), *D. samali* does not use its legs to elevate its body above the water during skimming locomotion (Fig. 1h–m); this posture causes the adult abdominal gills to be positioned at the air–water interface during rowing. The more posterior abdominal segments lack gills but instead have spiracles on the lateral ventral surface that appear to be submerged during rowing.

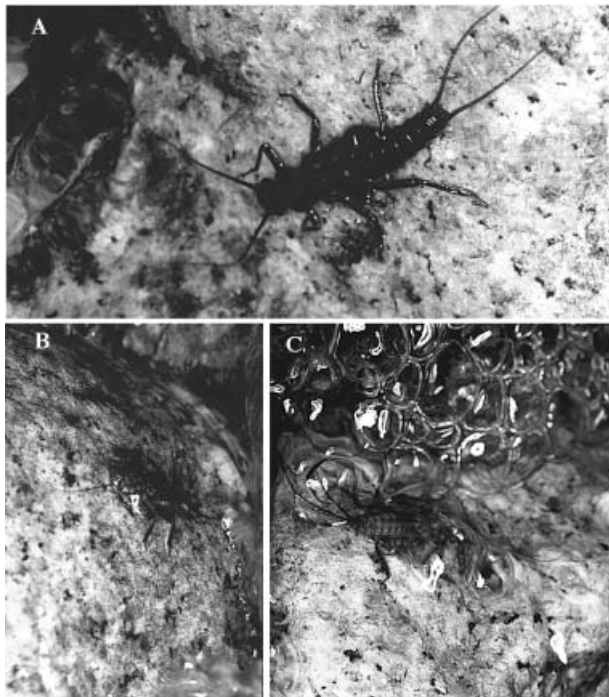


Figure 5. Photographs of foraging by final-instar *D. samali* nymphs in areas where they are in contact with both air and water. (A) Nymph feeding on algae that is just beneath the water surface (at approximately the level of the mesothoracic legs); the abdomen and cerci protrude above the water surface and are exposed to air. (B) Nymph feeding within a very thin film of water flowing over a rock. (C) Nymph feeding within a curtain of air bubbles formed by rapids. Algae grow particularly fast in these areas of relatively high temperature and oxygen.

Diamphipnopsis samali nymphs displayed behaviours that presented opportunities to accomplish both aquatic and aerial gas exchange. During daylight, nymphs remained hidden under rocks in shallow water near the lateral margin of the stream. At night, these nymphs emerged to feed on algae growing at the air–water interface, either at the stream margin (Fig. 5a) or within thin films or bubble curtains formed by rapids (Fig. 5b,c). Their abdominal gills were often exposed to air during these behaviours.

Haemolymph from *D. samali* stoneflies showed no reversible binding of oxygen, whereas haemolymph from a blue crab (our positive control) showed a typical oxygen dissociation curve (Fig. 6) for crustacean blood (e.g. Innes & Taylor, 1986). Using PCR primers for conserved regions of the arthropod haemocyanin and hexamerin gene family (designed using data from Burmester, 2001), we were able to amplify and sequence an 800 nucleotide fragment of a hexamerin gene from *D. samali* cDNA, but not a haemocyanin gene (Girgenrath & J. H. Marden, unpubl. data). Thus, we found no evidence that *D. samali* stoneflies possess a haemocyanin that reversibly binds oxygen.

DISCUSSION

Rowing behaviour of *Diamphipnopsis* stoneflies is a novel addition to the distinct forms of surface-skimming locomotion used by adult insects to move

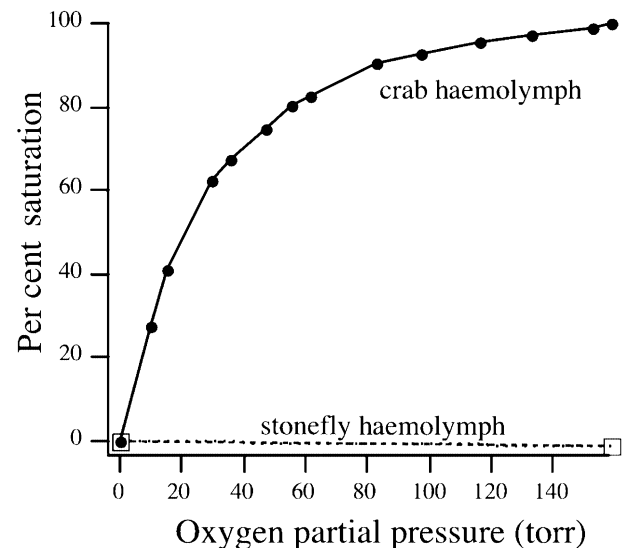


Figure 6. Oxygen binding curve for haemolymph from the blue crab, *Callinectes sapidus*. The spectrophotometer was zeroed at the absorbance of deoxy-haemolymph. For stonefly haemolymph, there was no change in absorbance at any oxygen partial pressure; for the sake of simplicity we show only the data for two end points.

across the surface of water. Surface skimming is mechanically diverse, taxonomically widespread and phylogenetically basal in the order Plecoptera (Marden *et al.*, 2000; Thomas *et al.*, 2000), and occurs sporadically in other relatively basal pterygote taxa such as mayflies (Ephemeroptera; Ruffieux, Elouard & Sartori, 1998) and damselflies (Odonata; Samways, 1994). What makes rowing especially interesting is that it demonstrates how a fairly simple form of cyclical wing motion can be used in a semi-aquatic setting to accomplish locomotion that is mechanically intermediate between gill-flapping and flying. Rowing locomotion provides a model for how certain early pterygotes with rudimentary wings and abdominal gills (Fig. 1a–d) might have behaved.

Diamphipnopsis stoneflies belong to the Eusthenioidea, a superfamily that morphological phylogenetic analysis has placed at the most basal branch of modern Plecoptera (Nelson, 1984). However, a molecular phylogeny using 18S rDNA sequence indicates that this group is relatively derived (Thomas *et al.*, 2000). Regardless of their phylogenetic status, species in the superfamily Eusthenioidea, and stoneflies in general, appear to have retained many plesiomorphic traits (Illies, 1965; Brodsky, 1994) and therefore may be informative regarding the physiology, behaviour and mechanics of certain lineages of early Pterygotes.

We looked for but did not find one potentially plesiomorphic trait, the presence of functional haemocyanin. A haemocyanin protein that contains all of the residues involved in oxygen binding, oligomerization and allosteric regulation of oxygen transport is expressed in at least one modern insect (an orthopteran; Sanchez *et al.*, 1998), but reversible oxygen binding by haemocyanin has not yet been demonstrated in any insect. Nonetheless, the presence of an expressed and potentially functional haemocyanin in a grasshopper makes it plausible that there was a partial reliance on blood-based gas exchange in early pterygotes, and this ability may persist in some modern species. A wider exploration of the oxygen binding capability of the haemolymph of relatively basal taxa of modern insects might reveal a partial role for blood-based oxygen transport. Such a finding would have a strong impact on hypotheses regarding the evolution of insect gas exchange.

Retention of gills by adult insects occurs sporadically among the basal pterygote orders (Štys & Soldán, 1980). Adult gills tend to be small, variable in size and number, and appear to be vestigial. However, the only European mayflies that retain substantial adult gills (the *Palingenia*; Štys & Soldán, 1980) are also the only European mayflies known to make extensive use of surface skimming in order to mate on the surface of water (see video sequences of *P. longicauda* at <http://www.famu.org/mayfly/sartori/movies.asp>).

Co-occurrence of adult gills and skimming locomotion on both branches of the basal radiation of Pterygota (i.e. the Palaeoptera, as represented by mayflies, and the Neoptera, as represented by stoneflies) supports the hypothesis that early pterygotes with rudimentary wings and abdominal gills (Fig. 1a–d) were surface skimmers, and retention of adult abdominal gills in multiple species of extant surface skimmers suggests that gills may serve an as yet unknown functional role during skimming.

Rowing by insects with abdominal gills provides a context in which gills and rudimentary wings could have coexisted, during a time when tracheae were perhaps gradually invaginating and becoming elaborated, and insect gas exchange was still at least partially blood-based (i.e. reversible oxygen binding by respiratory pigments). During rowing by *D. samali*, the abdominal gills are located in a position where they are in contact with both air and water. This position would have allowed structural specializations for aquatic respiration (elaborate branching and collapsibility) to be replaced gradually by structural specializations for aerial respiration, such as reduced branching and rigidity. Early pterygotes may have first begun to exploit the approximately 30-fold greater concentration of oxygen in air (Schmidt-Nielsen, 1990; this difference was probably even greater during the Carboniferous when atmospheric oxygen concentration was elevated; Dudley, 1998) by moving about on the water surface and/or margin in a habitat where their gills, both thoracic and abdominal, could remain moist and structurally supported. Both rowing by adults (Fig. 1) and foraging by *D. samali* nymphs (Fig. 5) at the surface and edges of their aquatic habitat provide a behavioural setting in which gills are exposed to both air and water. Gradual evolution of aeri ally adapted gills, followed by externally open tracheae that bypassed gill-based gas exchange altogether, may have freed the thoracic gills of early pterygotes to become specialized for novel functional roles such as rowing, sailing (Marden & Kramer, 1995), and ultimately flapping forms of aerodynamic locomotion (Marden *et al.*, 2000), while eventually rendering abdominal gills unnecessary for the adult stage.

This line of thinking can be combined with results from developmental genetics to form a synthetic hypothesis for the evolution of early pterygotes. The arrangement of limbs in the pterygote body plan is proposed to have evolved in a two-step process that involved changes in expression and sequence of the homeotic gene *ultrabithorax* (*Ubx*), which limits the expression of the limb-promoting gene *distal-less* (*Dll*). In the first step, *Ubx* expression became restricted to the abdominal region, and in the second step, mutations in *Ubx* pathways resulted in enhanced suppression of appendages on the abdomen (Averof & Akam,

1995). If we envision these genetic changes occurring during a transition from an aquatic lifestyle to semi-aquatic activity on the water surface, then relocation of the moderately limb-repressing effect of *Ubx* away from the thorax may have allowed enlargement of thoracic appendages and consequent enhancement of their locomotor function (i.e. a gills-to-oars-to-wings transition), and the fossils shown in Figure 1a–d may represent various parallel stages of such an evolutionary process. Subsequent mutations in the C-terminal region of *Ubx* that increase its limb repressive effect (Galant & Carroll, 2002; Ronshaugen *et al.*, 2002) may have then eliminated abdominal gills as the development of tracheae replaced their function. This scenario provides a functional framework for the hypothesis of Ronshaugen *et al.* (2002) that ‘successive removal of serine-threonine residues [from *Ubx* protein] might quantitatively influence repression function and morphology, allowing viable microevolutionary steps toward “hopeful monsters” with macroevolutionary alterations in body shape’.

Developmental genetic analyses and sequencing of *Ubx* have not yet examined relatively basal pterygotes such as mayflies and stoneflies that possess abdominal gills. These taxa are likely to have intermediate forms of the limb-repressive C-terminal domain of *Ubx*, and different orders may show a diversity of *Ubx* sequence and function because the repression of abdominal gills must have evolved independently (i.e. after divergence of the taxa shown in Fig. 1). Developmental genetic studies of the basal pterygote orders may reveal various intermediate stages of homeotic evolution and repression of abdominal appendages, and may shed additional light on the early evolution of flying insects.

ACKNOWLEDGEMENTS

We thank B and G. Aldrich for hospitality and logistical assistance during field work in Chile, O. Flint for providing specimens that sparked our interest in *Diamphipnopsis*, J. Bye for drawings, and R. Walsh for electron microscopy, and three reviewers for their constructive comments on the manuscript. This project was supported by NSF grant IBN-9722196.

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