

## Locomotor performance of insects with rudimentary wings

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THE evolution of flight in insects triggered an unparalleled radiation and diversification such that flying insects comprise approximately two-thirds of all species<sup>1</sup>, yet a gap in the fossil record obscures the origins of wings and flight<sup>2</sup>. Among modern insects, stoneflies are morphologically primitive for several flight-related traits, which makes their locomotor behaviour and physiology of particular interest<sup>3</sup>. Here we show that *Allocaupnia vivipara* stoneflies use a non-flying form of aerodynamic locomotion which may exemplify a precursor to flight. They raise their wings in response to wind, thereby sailing across water surfaces, but they are incapable of flapping. Sailing performance improves steadily with increasing wing size, and even the smallest wings significantly increase sailing velocity compared to wingless individuals. Performance during aerial gliding is less affected by wing size, which suggests that sailing is a more plausible setting for wing evolution. These results support the hypothesis that insect wings evolved from articulated gill plates of aquatic ancestors through an intermediate semi-aquatic stage<sup>4</sup>.

Previous studies of insect flight evolution have assumed that aerial gliders preceded powered fliers<sup>2,3,5,6</sup>. However, the preponderance of evidence indicating that wings evolved from articulated gill plates used by aquatic forms for ventilation and/or locomotion<sup>7,8</sup> suggests that the potential of proto-wings to enhance semi-aquatic locomotion should be explored.

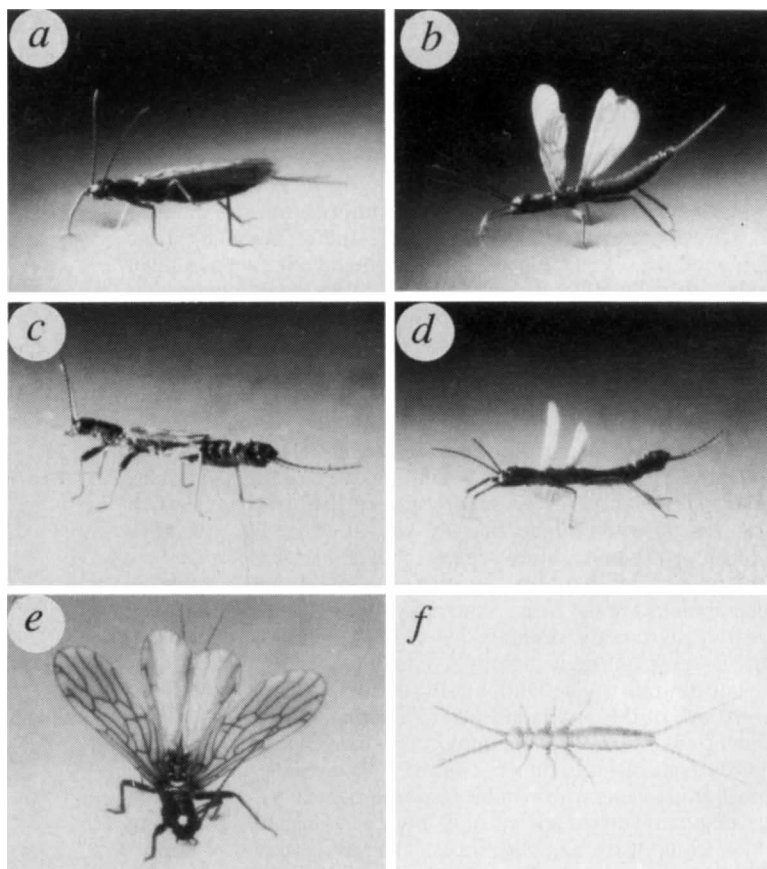
Accordingly, we recently studied a stonefly (*Taeniopteryx burksi*; Plecoptera: Taeniopterygidae) that uses wing-flapping locomotion to move on the water surface<sup>4</sup>. Surface-skimming is effective even when wing size is greatly reduced and muscle power output is minimized. Here we extend that study by examining aerodynamic performance of a non-flapping stonefly whose anatomy, physiology and behaviour may even more closely approximate that of the first winged insects.

In the northeastern United States, *Allocaupnia vivipara* stoneflies (Plecoptera: Capniidae) emerge as adults from stream-dwelling aquatic nymphs during mid-January through to April. Females are uniformly long-winged (70–105% of body length), whereas males are mostly short-winged (33–65% of body length). Individuals that emerge as adults on midstream rocks, sticks or ice stand on top of the water surface tension and raise their wings in response to gusts of wind (Fig. 1), thereby sailing across the surface and reaching shore at locations where the wind is perpendicular to the direction of current flow. Even at warm laboratory temperatures, these stoneflies never flap their wings.

We built a wind tunnel over a water surface to examine the effect of wing size on sailing performance. At all wind speeds tested, sailing velocity improved linearly with increasing wing length (Fig. 2), and stoneflies with the smallest wings had significantly improved sailing performance compared to 'wingless' individuals (those that did not raise their wings). Relative wing length of *A. vivipara* males (wing length/body length) is as low as 0.33, a value similar to the size of articulated, moveable gill plates of early pterygote insect nymphs from the Carboniferous and Permian<sup>7</sup>. Thus gill plates of protopterygotes might have been useful as sails if these insects were active on the water surface, as many extant apterygote insects are<sup>9</sup>, and raised their gill plates in response to wind.

*Allocaupnia* stoneflies also raise their wings and glide when they are dropped into air (a behaviour that appears to be used only rarely in nature), and thus it is possible to compare the func-

FIG. 1. Surface sailing in the laboratory in *A. vivipara* stoneflies. *a*, A female walking on the surface of water, and *b*, after raising her wings in response to gentle wind. *c*, A male walking on water, and *d*, after raising his wings in response to wind. Note the stereotypic leg posture of the stoneflies with raised wings. This leg stance provides a wide and stable base during sailing. *e*, Head-on view of a female with raised wings. This particular photograph was taken in still air; the stonefly is walking and periodically raising its wings, presumably in response to very small air currents. Although the leg posture is atypical, the wing posture nicely shows the 'spinnaker sail' arrangement of the wings. Video segments in platform-independent QuickTime format can be obtained through the World Wide Web (<http://cac.psu.edu/~jhm10>). *f*, Drawing of an apterygote insect (Diplura; Campodeidae) to show the gross morphological similarity of sailing stoneflies and primitively wingless insects.



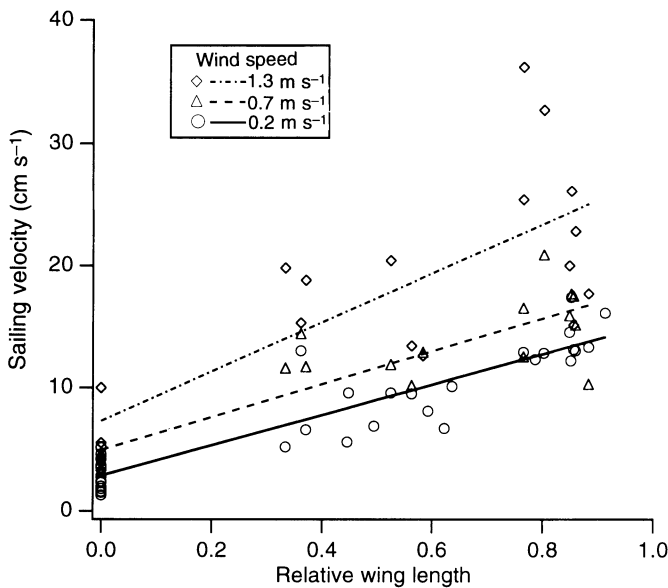


FIG. 2 Sailing velocity of *A. vivipara* stoneflies as a function of relative wing length (forewing length/body length). Each data point represents a different individual. Data from wind speeds of 0.5 and 1.0  $\text{m s}^{-1}$  ( $N=4$  for each wind speed) were too few to establish a regression line; these points have been omitted to improve clarity of the figure. Data points at a wing length of zero represent individuals that did not raise their wings in response to wind, thus approximating the aerodynamic performance of wingless stoneflies. Multivariate regression shows that forewing length (partial  $r^2=0.57$ ) and wind speed (partial  $r^2=0.20$ ) contributed significantly ( $P<0.0001$ ;  $N=79$ ) to sailing velocity, whereas body length did not ( $P=0.74$ ).

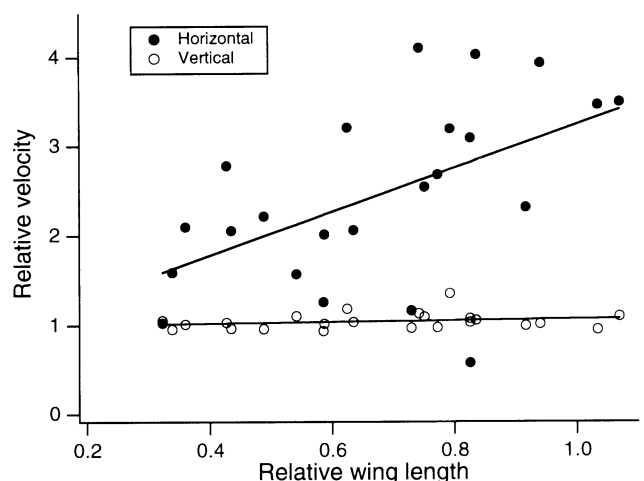
tional advantage of wings for sailing versus gliding. We blew gently on *A. vivipara* stoneflies to stimulate and verify wing raising, then dropped them into still air above a motion-tracking device that recorded their three-dimensional glide path. Each individual was subsequently tested after wing removal. Relative horizontal velocity (velocity with wings/velocity without wings) increased with increasing wing size (Fig. 3). On average, the smallest wings yielded a 1.7-fold improvement in relative terminal horizontal velocity compared to the same individuals after wing clipping. Relative terminal vertical velocity was not affected by wing size.

Relative horizontal velocity was higher for surface sailing than for aerial gliding at all relative wing sizes (Fig. 4), and gliders needed wings 80% as long as their body before they reached the level of increase in relative performance achieved by sailers with the smallest relative wing size (33% of body length). This difference probably stems from the fact that sailing stoneflies maintain a nearly constant and perhaps optimal body orientation with respect to the wind (Fig. 1*b,d,e*), whereas stoneflies in air tend to tumble, with their wings often in a poor position to produce beneficial aerodynamic forces. Thus these data suggest that sail-

ing has a greater potential to drive the evolution of insect wings than does aerial gliding.

An early trend in insect wing evolution was an elaboration of thoracic winglets, accompanied by a reduction and eventual loss of serially homologous abdominal winglets<sup>7,8</sup>. The loss of abdominal winglets is difficult to explain in the context of aerial gliding or thermoregulation, for all lateral projections from the body would have contributed to both gliding performance and solar energy capture<sup>5,6,10</sup>. In contrast, the surface-sailing hypothesis offers a clear explanation for abdominal winglet reduction, based on the effect of abdominal winglets on body orientation. The centre of rotation for a surface sailing insect is located approximately midway between the thoracic wing bases. Aerodynamic forces on thoracic wings would cause little net rotational moment, but forces on abdominal winglets located posterior to the centre of rotation would have a large rotational moment, causing the body to rotate head-on into the wind. In such a position (Fig. 1*e*), abdominal winglets would be downwind of thoracic winglets; they would not increase the aerodynamically functional surface area or contribute significantly to sailing performance.

FIG. 3 Relative velocity (mean terminal velocity with wings/mean terminal velocity without wings, measured from 2–6 tests for each individual before and after the wings were removed completely by clipping) as a function of relative wing length during aerial gliding by *A. vivipara* stoneflies ( $N=23$ ; each data point represents one individual). Wing length had a significant effect on horizontal velocity ( $r^2=0.32$ ;  $P=0.005$ ) but not on vertical velocity ( $r^2=0.02$ ;  $P=0.53$ ). Absolute terminal vertical velocity varied significantly with body mass ( $r^2=0.56$ ;  $P<0.0001$ ) according to the equation: velocity ( $\text{m s}^{-1}$ ) =  $-0.072$  body mass (mg)  $-1.25$ . Absolute terminal horizontal velocity varied significantly ( $r^2=0.57$ ;  $P=0.0007$ ) with wing length according to the equation: velocity ( $\text{m s}^{-1}$ ) =  $0.043$  wing length (mm)  $-0.015$ . Mean body mass and length of *A. vivipara* stoneflies was 2.39 mg (range, 0.7–5.9 mg) and 4.8 mm (range, 2.4–7.4 mm). Aerial motion through a 10 cm  $\times$  8 cm  $\times$  8 cm airspace was tracked in 3 dimensions with a MacReflex motion analysis system (Qualysis Inc., Glastonbury, CT; 30,000  $\times$  30,000 pixel sensor grids; 60 Hz sampling) modified for 'inverse video', in which the centroid of a dark object (an insect that has not been marked or otherwise modified) is detected as it moves in front of a bright background (fluorescent lights diffused by white Plexiglas). Stoneflies were dropped from a height 15 cm above the calibrated airspace, and they reached terminal velocity before or shortly after entering the airspace through which they were tracked.



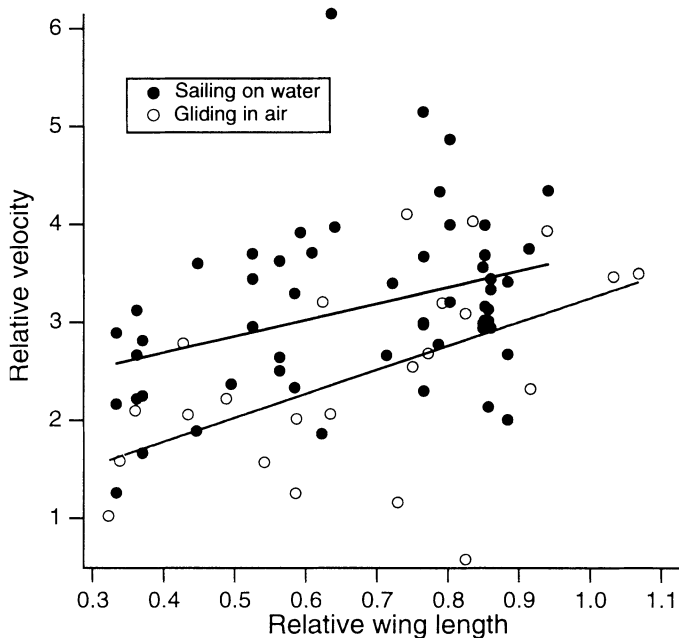


FIG. 4 Relative horizontal velocity (velocity with wings/velocity without wings) for *A. vivipara* stoneflies sailing across water and gliding horizontally through air, as a function of relative wing length (wing length/body length). Relative velocity for sailing was estimated by dividing the sailing velocity of each wing-raising individual (Fig. 2) by the velocity predicted from a multivariate model (independent variables are body mass, body length and wind speed;  $r^2=0.46$ ;  $P=0.005$ ) based on sailing velocity of the 24 individuals that did not raise their wings (data points at zero wing length in Fig. 2). Analysis of covariance indicates that the performance benefit of wings used for sailing is significantly greater than that of wings used for aerial gliding ( $P=0.0013$ ; covariate equals relative wing length).

The apparent homology of wing venation and wing articulation among all winged insects<sup>7,8</sup> has been interpreted as powerful evidence that insect flight evolved only once<sup>3,11,12</sup>, but the use of wings for non-flying surface locomotion shows that wings and flight are not necessarily coupled evolutionarily. Thus evolutionary radiation of early surface-locomotors may have preceded the attainment of powered flight, and therefore flight could have evolved several times, even though all flying insects share the same basic wing venation and articulation. Given this possibility, stoneflies may have retained or reverted to primitive locomotory behaviour and capacity in a form that is little changed from ancestral insects that first evolved wings a third of a billion years ago. At the very least, surface locomotion in stoneflies demonstrates how morphologically primitive insects can use rudimentary wings and wing motions to accomplish aerodynamic locomotion, which in turn suggests new ways to interpret fossils and the evolutionary history of winged insects. □

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## Unusual thermal defence by a honeybee against mass attack by hornets

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THE giant hornet *Vespa mandarinia japonica* (Hymenoptera: Vespidae) is the only hornet species known to have evolved *en masse* predation of other social bees and wasps. Here we show that hornets initiate mass attack by secretion of a foraging-site marking pheromone from the van der Vecht glands (metasomal sternum VI glands) by a single foraging hornet. The lone hornet rubs the basal tuft of the terminal gastral sternite around a prey food resource, such as a honeybee colony, and the hornet nestmates then congregate and attack the marked site *en masse*. The sympatric Japanese honeybee *Apis cerana japonica* (Hymenoptera: Apidae) can detect the hornet marking pheromone, and responds by increasing the number of defenders at the nest entrance. When an invading hornet is captured by a defending bee, more than 500 other bees quickly engulf the hornet in a ball which contains isoamyl acetate. Thermography showed that the ball temperature is very high (~47 °C), which proves lethal to the hornet but not to the bees. Defenders patrolling the nest entrance also generate high temperatures. These findings suggest that aspects of the interaction between *V. mandarinia japonica* and *A. cerana japonica* are specifically coevolved.

The Japanese giant hornet is a major predator of social bees and wasps. Unlike the Japanese honeybee, colonies of the introduced European honeybee *Apis mellifera* are quickly destroyed by mass attacks of the hornet<sup>1</sup>, because the giant hornet is allopatric and so the European honeybee has not evolved an effective defence. Earlier observations of the defensive behaviour of the Japanese honeybee suggested that the bees sting the hornet to death<sup>2</sup>, but during our study in Tokyo from 1984 to 1994 we found no evidence of stinging when killed hornets were investigated.

The foraging sequence of the giant hornet exhibits several distinct phases<sup>2</sup>. First, a lone foraging hornet finds the honeybee nest, kills individual bees and takes them to its nest to feed to the larvae (hunting phase). After several return visits, the foraging hornet marks the site by rubbing its van der Vecht gland on or near the bee colony (recruitment phase; Fig. 1a). Some polistine wasps (*Polistes*, *Mischocyttarus*, *Parapolybia* and some *Ropalidia*) are known to secrete ant repellent from the van der Vecht gland<sup>3,4</sup> (Fig. 1b), but this is not the case with *Vespa*.

Soon after marking, nestmate hornets flying in the same area congregate at the marked site and start individual hunting. Their hunting behaviour suddenly changes when there are three or more, and they attack *en masse* (slaughter phase). One hornet can kill up to 40 European honeybees per minute with its mandibles, and a colony of 30,000 bees can be killed in 3 hours by a group of 20–30 hornets, which then occupy the hive (occupation phase). During this occupation period, which lasts more than 10 days, the hornets carry bee larvae and pupae to their nest as food for their larvae.

Unlike the European honeybee, the Japanese honeybee has an effective defence against hornet attack. Instead of counter-attacking the hornet individually, more than 100 workers crawl around the nest entrance (Fig. 1c). When a hornet approaches, they simultaneously lift and shake their abdomens and then escape into the nest, and more than 1,000 workers leave the comb and wait just inside the entrance. If a foraging hornet tries