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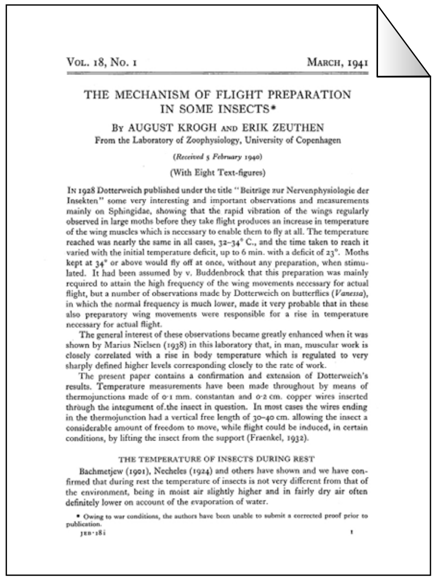
THE ORIGIN OF INSECT THERMOREGULATORY STUDIES



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THE MECHANISM OF FLIGHT PREPARATION IN SOME INSECTS*

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(With Eight Text-figures)

IN 1928 Dotterweich published under the title "Beiträge zur Nervenphysiologie der Insekten" some very interesting and important observations and measurements mainly on Sphingidae, showing that the rapid vibration of the wings regularly observed in large moths before they take flight produces an increase in temperature of the wing muscles which is necessary to enable them to fly at all. The temperature reached was nearly the same in all cases, 32–34° C., and the time taken to reach it varied with the initial temperature deficit, up to 6 min. with a deficit of 23°. Moths kept at 34° or above would fly off at once, without any preparation, when stimulated. It had been assumed by v. Buddenbrock that this preparation was mainly required to attain the high frequency of the wing movements necessary for actual flight, but a number of observations made by Dotterweich on butterflies (*Vanessa*), in which the normal frequency is much lower, made it very probable that in these also preparatory wing movements were responsible for a rise in temperature necessary for actual flight.

The general interest of these observations became greatly enhanced when it was shown by Marius Nielsen (1938) in this laboratory that, in man, muscular work is closely correlated with a rise in body temperature which is regulated to very sharply defined higher levels corresponding closely to the rate of work.

The present paper contains a confirmation and extension of Dotterweich's results. Temperature measurements have been made throughout by means of thermojunctions made of 0·1 mm. constantan and 0·2 cm. copper wires inserted through the integument of the insect in question. In most cases the wires ending in the thermojunction had a vertical free length of 30–40 cm. allowing the insect a considerable amount of freedom to move, while flight could be induced, in certain conditions, by lifting the insect from the support (Fraenkel, 1932).

THE TEMPERATURE OF INSECTS DURING REST

Bachmetjew (1901), Necheles (1924) and others have shown and we have confirmed that during rest the temperature of insects is not very different from that of the environment, being in moist air slightly higher and in fairly dry air often definitely lower on account of the evaporation of water.

* Owing to war conditions, the authors have been unable to submit a corrected proof prior to publication.

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Bernd Heinrich writes about August Krogh and Eric Zeuthen's 1941 classic paper on insect thermoregulation entitled 'The mechanism of flight preparation in some insects'. A copy of the paper can obtained at http://jeb.biologists.org/cgi/reprint/18/1/1

Some 'classic' papers shine for their sheer brilliance and thoroughness. They put an end to argument. Others pioneer a new method that opens up novel directions of research, or they focus on a previously ignored work and bring it to light. Still others have impact because they draw attention when a big gun stumbles across an obstacle or exposes a gaping hole in our knowledge. I believe that the 1941 paper by August Krogh and Eric Zeuthen does some or all of the above in the area where insect physiology intersects ecological energetics and thermoregulation. Working together, they examined a butterfly, a bumblebee, and a beetle, and concluded that the temperature of an insect's flight muscle during pre-flight warm-up determines its maximal rate of work output during flight (Krogh and Zeuthen, 1941). I first read the Krogh and Zeuthen paper in the mid 1960's when I became interested in insect physiology with the aim of discerning mechanisms of thermoregulation. I think their paper was inspiring, not for any one particular discovery, but rather for their approach.

However, the concept that flight muscle activity raised body temperature was hardly new, even in 1941. A century earlier George Newport had reported that there is a correlation between activity and elevated body temperature in a moth, a bumblebee, and a beetle (Newport, 1837). After the subject remained fallow for the following 60 years the Russian physicist Perfirij J. Bachmetjev resurrected the subject when he identified the same correlation in insects just before the end of the 19th Century (Bachmetjev, 1899). Similarly, Heinz Dotterweich showed specifically that the rise in thoracic temperature of sphinx moths is related to the insects' flight preparations (Dotterweich, 1928). In Krogh's own laboratory in Denmark, Marius Nielsen showed that human body temperature also rises during strenuous activity, and is then regulated at a high level corresponding to work output (Nielsen, 1938). Referencing these early, possibly forgotten, classical studies in Krogh and Zeuthen's 1941 paper brought the neglected topic of thermoregulation to the forefront of the then hot field of respiratory physiology.

Prior to Krogh and Zeuthen's work, reports of insect thermoregulation were mainly descriptive. However, their 1941 paper was the first to attempt to crack the proverbial black box of the underlying physiological mechanisms. It set the stage for subsequent work by reviewing salient points from the scant data available on muscle temperature and mechanical work of insects prior to flight or while resting. Using thermocouples implanted in butterfly (*Vanessa*) flight muscles, they demonstrated that wing movements during both pre-flight shivering and flight, are associated with a steady rise in muscle temperature until temperatures approaching human body temperature are reached. The butterflies were then thrown into the air to find the muscle temperatures that enabled the insects to fly. Krogh and Zeuthen's observation led them to disagree with previous observations about insect flight temperatures, stating that '*We cannot subscribe to Dotterweich's statement that moths require a definite temperature to be able to fly. We made a few observations on Catocola sponsa, measuring thoracic temperature and then throwing the moths into the air. These observations indicate that at muscle temperatures above 25°C this species is able to fly*'. Although Krogh and Zeuthen's statement does not specify what temperatures 'a few' observations encompass, nor what a 'definite' temperature is, it is clear to me that the moth can fly at temperatures as low as 25°C and is not restricted to just the narrow range of high muscle temperatures that they reported for flight in other insects. Apparently Dotterweich drew incorrect generalizations about temperature regulation, extrapolating from sphinx moths to other moths.

Next the team extended their observations of flight muscle temperature into the bumblebee *Bombus horti* and found that the bumblebee's temperatures paralleled those of the butterfly; the thoracic muscles heated up to at least 30°C before flight. Measurements of the insect's abdominal temperature showed that it was only slightly elevated, enhancing the team's point that the flight muscles are indeed the source of the body heat, and that the action of warming-up permits high energy expenditure during flight.

Krogh and Zeuthen's final observations in their 1941 paper focused on the large (weighing in at almost one gram) lamellicorn beetle, *Geotrupes stercorarius*. Large beetles 'pump' their abdomen prior to flight, and since beetles show no externally-visible motion of the wings or elytra prior to flight, it was presumed that they did so in order to raise the oxygen concentration of the tracheal system. However, Krogh and Zeuthen's electrical recordings from the flight muscles showed neural spiking activity as the beetle warmed up; the flight muscles were active even though the wings did not move. That is, these insects, which appeared to fly without prior shivering, were indeed exercising their flight muscles, and since working muscles require vigorous gas exchange, that explained the abdominal pumping. It was this observation of 'invisible' muscle activity in particular that I found the most intriguing, because it showed that much was still hidden and unknown.

For me, one of the most provocative aspects of Krogh and Zeuthen's paper was their use of the insects' cooling curves to estimate energy expenditure, measurements which are still considered virtually impossible in free-living animals. When their insects stopped exercising they cooled rapidly to their initial body temperatures, and from the cooling curve Krogh and Zeuthen calculated the insects' energy expenditures, compared them with insect's metabolic rate calculated from measured rates of carbon dioxide production, and found 'satisfactory agreement'. Furthermore, they converted metabolic rates to the caloric intake from sugar collected from flowers that was required to support the insects in pre-flight preparation.

Given their simple calculations, Krogh and Zeuthen concluded that the heating process during insect pre-flight warm-up is unlikely to be an adaptation for the discharge of nervous impulses from the ganglia to the muscles. Instead, they state that it is 'required to allow the muscular engine to develop the energy expenditure for flight'. This, a major point of their paper, established the framework and a trajectory of subsequent insect thermoregulation studies for those that followed their lead into physiology. To my knowledge, the authors themselves did not proceed further in this area, possibly because of the war: there is a note at the end of the paper, which states that '*Owing to war conditions, the authors have been unable to submit corrected proofs prior to publication*'.

While the paper clearly laid out the essential role of thermoregulation in flight, the authors also enunciated several apparent enigmas that would concern many of the researchers who followed in their wake. For example, why do some insects require a high muscle temperature in order to fly, while others do not? Krogh and Zeuthen assumed (an assumption that held for the next 30 years) that the maximum flight temperature achieved is only that which the insect spends valuable energy to achieve. Since all the work was done with highly restrained animals, not free-flying ones, there was never any suggestion that some insects might produce heat in excess of their flight requirements. In their attempt to explain this enigma of variable flight temperatures, Krogh and Zeuthen merely suggested that those insects requiring high muscle temperatures are 'bad flyers' and those who fly at lower temperatures 'good flyers'. It would nowadays be a bit of a stretch to characterize sphinx moths and bees as 'bad' flyers. Undoubtedly, many subsequent studies on the aerodynamics of insect flight owe at least some of their inspiration to Krogh and Zeuthen's claim about the bumblebee's ineptitude. I was personally inspired to instigate numerous studies to determine what body temperatures insects flew with and why some had evolved to fly with a low but others with a high thoracic temperature, all of which ultimately provided insights into the evolution of thermoregulation (Heinrich, 1977).

The authors also made mistakes. They incorrectly posited from their electrical recordings from bumblebee flight muscles that the muscles of these bees generated a vibration frequency of 100 Hz during shivering (*vs* 20 Hz for Vanessa butterfly flight muscle). They had apparently assumed that the bee was shivering only when it moved its wings to buzz, making the 'reasonable' assumption that there is a one-to-one correlation between action potentials and wing muscle contractions that would translate to wing movements. These incorrect assumptions stimulated my own work when I realised that bees achieve impressive temperature increases without moving their wings, and it eventually became apparent that the wing muscles are in tetanus during warm-up (Kammer and Heinrich, 1974). Numerous subsequent studies over the next half century revealed fascinating mechanisms of muscle function and morphological adaptations for damping thoracic and wing vibrations during warm-up (Esch et al., 1991) and that 'warm-up' plays a role in a variety of other physiological phenomena besides flight preparation, including brood incubation and colony defense. Krogh and Zeuthen also implied that abdominal temperature is passive, with thoracic heat simply diffusing into the abdomen, setting up another strawman that stimulated subsequent research that ultimately yielded breakthroughs in our understanding of insect thermoregulation, behavior and social ecology (reviewed in Heinrich, 1993; Heinrich, 1996).

Their short (it would fit into 4 or 5 pages in JEB's current format) paper's main influence, I believe, arose not only from the clear and incisive insights it provided through simple direct observations, but also from the unknowns (and interesting mistakes) it highlighted. It also emphasises the little that was known about insect thermoregulation in 1941, most of which had been buried in the literature for up to a century.

Ironically, although the paper by Krogh and Zeuthen focused on muscle physiology, the last paragraph introduced a way of connecting the muscle temperature of the animal with practical estimates of its energy input and expenditure. For me, that observation culminated in field studies that revealed the ecological and evolutionary relationships between bees and flowers and stimulated me to write the book Bumblebee Economics (Heinrich, 1979), which encompassed the enigmas first hinted at in this classic paper and the insights they subsequently inspired.

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