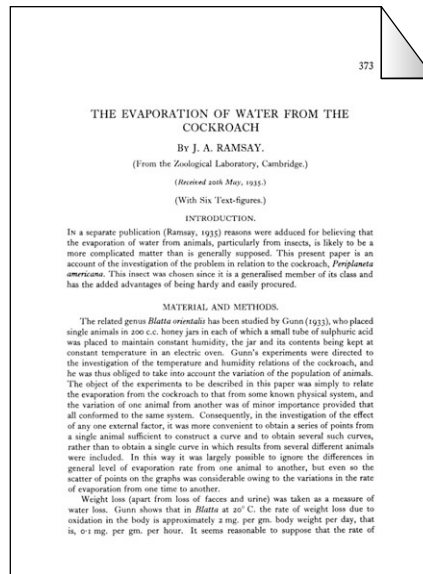


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# JEB CLASSICS

## WATERPROOF COCKROACHES: THE EARLY WORK OF J. A. RAMSAY



Allen Gibbs writes about J. Arthur Ramsay's 1935 classic paper on insect water balance entitled 'The evaporation of water from the cockroach'. A copy of the paper can be obtained at <http://jeb.biologists.org/cgi/reprint/12/4/373>

The relatively small size of insects, and thus their high surface area-to-volume ratio, implies that water balance is a central issue in insect physiology. Many of the most important papers on the topic have appeared in *The Journal of Experimental Biology*, perhaps partly due to the long co-editorship (1955–1974) of Sir Vincent B. Wigglesworth and J. Arthur Ramsay. Several years ago, we used Wigglesworth's 1945 JEB paper in a graduate seminar on 'Classic Papers in Comparative Physiology', publications that broke new ground and set the direction for research in their disciplines. Even classic studies have their antecedents, however, and Wigglesworth (Wigglesworth, 1945) clearly drew upon Ramsay's earlier work (Ramsay, 1935b).

This JEB classic was actually the second of a pair, Ramsay's first publications in a 40-year career (Ramsay, 1935a; Ramsay, 1935b). The first and longer paper considered the theory of evaporation from surfaces, rigorously evaluated previous work on evaporation from animals, and described Ramsay's own experiments on evaporation from a 'model animal', in this case a porous pot. Although Ramsay considered this paper to be of higher quality (Maddrell, 1998), it is the second paper that has become a classic.

Ramsay then used the same apparatus to investigate water loss from an actual animal: the American cockroach, *Periplaneta americana* (Ramsay, 1935b). Roaches were mounted in a thermostatted wind tunnel, and water loss was estimated from mass loss. He also made a model insect from ebonite, an early plastic, complete with a 'tracheal system' drilled into it. The lowest flow rates used ( $18 \text{ km h}^{-1}$ ) were strong enough to disperse any boundary layer, so one would expect water loss to be independent of air speed. It was thus surprising that very high wind speeds significantly increased rates of water loss. Ramsay attributed this result to eddies set up within the tracheal system, increasing water evaporation from inside the tracheal system itself. This was perhaps not so surprising when one considers that the higher air flows ranged up to  $>70 \text{ km h}^{-1}$ .

What makes this paper a classic? Using his own results and data re-plotted from Gunn (Gunn, 1933), Ramsay provided evidence for what was later termed the critical or transition temperature for water loss. Water-loss rates increase rapidly above this temperature, and the phenomenon has been reproduced *ad nauseam* in a wide variety of insects. What is the mechanistic basis for the transition temperature? Again, Ramsay provides the first evidence (Ramsay, 1935b). His 'incautious use of a tap' (Maddrell, 1998) sprayed a specimen with water, and drops settling on the cockroach remained intact long after nearby drops on the experimental apparatus disappeared. Further investigation revealed that these drops were coated with a film that he deduced was composed of lipids. Waxy substances in insect cuticle had been described (Kühnelt, 1928; cited by Wigglesworth, 1933), but their composition and function was not clear. Ramsay then performed the first biophysical measurements on surface waxes, finding that the surface tension of wax-coated droplets decreased dramatically at about  $30^\circ\text{C}$ , right where water loss began to increase. He concluded that melting of these same lipids on the cockroaches' cuticle was responsible for increased transpiration.

Although almost any insect or comparative physiology textbook will discuss critical temperatures and the role of lipid melting, Ramsay's findings were not immediately appreciated. Ramsay himself did not follow up on this work; indeed, he never cited it in 40 years of subsequent research on osmoregulation and water balance. It was not until Wigglesworth and Beament's 1945 papers (in *The Journal of Experimental Biology*, of course) that physiologists began to take notice. Beament used capillary melting point methods, a model membrane preparation, and contact angle measurements of water droplets to extend Ramsay's results to additional species (Beament, 1945). Since

then, every 10–15 years someone has come along with a new biophysical approach (e.g. Holdgate and Seal, 1956; Lockey, 1976; Toolson et al., 1979; Machin and Lampert, 1990; Gibbs and Crowe, 1991). None of these has seriously challenged Ramsay's original conclusion that the melting of the surface lipids directly affects cuticular transpiration.

Ramsay essentially got the critical temperature story right the first time, although an element of luck was involved. The cuticular lipids of most insects are dominated by long-chain hydrocarbons (although perhaps this impression is due to the relative ease of characterizing these compounds), which do not form continuous monolayers on water surfaces. Although most of the surface lipids of *P. americana* are hydrocarbons (Gilby and Cox, 1963), this species is unusual in that it also has a relatively large fraction of fatty acids and aldehydes, which can form relatively impermeable monolayers (Lockey, 1976). Had he used almost any other insect, the water droplets would not have been coated with lipids, and Ramsay's work might well have been forgotten. Cockroaches have since earned a reputation for aberrant behavior in terms of cuticular properties; for example, Neil Hadley once told me he would never allow experimentation on a cockroach in his laboratory.

Where did Ramsay go from here? He published only two more papers on water loss (from onychophorans and leaves) before turning his attention to neuromuscular physiology and osmoregulation. The latter effort was more successful, including important work on Malpighian tubule function and water vapor uptake by mealworms. Along the way, he made major contributions to analyses of small-volume samples using nanoliter osmometers and flame photometry, wrote a highly-regarded textbook (*A Physiological Approach to the Lower Animals*, Cambridge University Press, 1952), and began co-editing *The Journal of Experimental Biology* with Sir James Gray in 1952.

Although lipid melting behavior and cuticular permeability of insects are staples of physiology textbooks, interest in surface lipids has shifted to chemical ecology. As gas chromatographs became available in the 1960s and 1970s, chemists began to probe the enormous diversity of insect waxes. It became apparent that these molecules serve important functions in intra- and inter-specific chemical communication (Howard, 1993), and identifying bioactive compounds, potentially applying them for pest control, has become an important area of research. It is tempting to speculate that the dual roles of cuticular lipids in communication and water balance will interact in interesting ways, but unfortunately

physiologists and chemical ecologists have tended to ignore each others' work.

In a striking example of convergent evolution, most terrestrial organisms have similar lipid waterproofing layers (Hadley, 1989; Wertz and van den Bergh, 1998; Riederer and Schreiber, 2001), and the same principles of lipid structure and barrier function apply. Biophysical studies in plants and vertebrates have become much more advanced than those performed with insects, for example revealing substantial meso-scale structuring of lipid layers (Bouwstra et al., 1995; Müller and Riederer, 2005). These structures can significantly alter permeability to water and other compounds, such as herbicides and topical medicines (Bunge et al., 1999). Presumably insect waxes share this heterogeneity (Gibbs, 2002), but insect studies lag far behind. This has been attributed to the difficulty of working with such small animals, but perhaps the excellence of Ramsay's original work is partly to blame. Although several topics in cuticular permeability later became controversial, for example Beament's monolayer model for molecular packing of cuticular lipids (Beament, 1958), the thermodynamics of diffusion through the cuticle (Toolson, 1978), and the proper way to plot data [linear versus Arrhenius plots (Machin and Lampert, 1989)], Ramsay's basic conclusions did not. Because Ramsay was so accurate in his initial observations and interpretation, his work never generated the controversy that might have sparked a greater volume of subsequent effort.

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