A perspective on insect water balance

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ABSTRACT

Insects have a large ratio of surface area to volume because of their small size; thus, they face the potential for desiccation in the terrestrial environment. Nonetheless, they constitute over half of identified species and their success on land can be attributed, in part, to adaptations that limit water loss and allow for effective gains of water from food, fluids or atmospheric water vapour. Reduction of water loss from the gut involves sophisticated mechanisms of ion recycling and water recovery by epithelia of the Malpighian tubules and hindgut. Water loss across the body surface is greatly reduced by the evolution of very thin but highly impermeable lipid-rich layers in the epicuticle. Respiratory water loss can be reduced through effective spiracular control mechanisms and by mechanisms for convective rather than diffusive gas exchange. In addition to extracting water from food sources, some insects are capable of absorption of atmospheric water vapour through processes that have evolved independently in multiple groups.

KEY WORDS: Malpighian tubule, Cuticle, Hindgut, Respiration, Water vapour absorption

Introduction

Over half of identified metazoan species are insects (Mayhew, 2007). However, as a primarily terrestrial group, insects are at a physiological disadvantage because their small size and associated large ratio of surface area to volume create the potential for rapid water loss. The potential for desiccation in the terrestrial environment can be appreciated by comparing the impact of equilibrium humidity (75% relative humidity, RH) in the air above a solution of saturated NaCl ($\sim$6 mol l^{-1}) on different animals. Large animals, such as humans, would consider air at 75% RH to present an uncomfortably high level of humidity at temperatures above approximately 25°C; yet, to insects, this relative humidity poses a desiccation stress equivalent to that experienced by an aquatic species exposed to a solution about 12 times as concentrated as sea water (Winston and Bates, 1960). One factor in the success of insects as terrestrial organisms is their impressive ability to maintain water balance despite this potential for dehydration. Maintaining water balance requires insects to be extremely efficient in limiting water loss from the gut, the body surface and the respiratory system, as well as in gaining water from sources in food and even the atmosphere (Fig. 1). Adaptations that have been reviewed elsewhere and not considered in this Commentary include entry into dormant stages to avoid periods of restricted water availability in hot or cold environments (Benoit, 2010), anhydrobiosis (Sogame and Kikawada, 2017) or storage of water

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bound to carbohydrates (Djawdan et al., 1998) or in reservoirs such as the haemolymph (Folk and Bradley, 2005).

Water loss from the excretory system

Insect excretory systems provide examples of an extraordinary capacity to either conserve water or eliminate it when present to excess. Whereas some insect species such as the mealworm beetle *Tenebrio molitor* can complete their life cycle with access only to dry food and no liquid water (Machin, 1975), fluid-feeding species ingest prodigious amounts of water and produce correspondingly large volumes of urine. Larval aphids, for example, eliminate urine at rates exceeding their own body mass each hour when feeding (Fischer et al., 2002). The blood feeder *Rhodnius prolixus*, a vector for Chagas disease, ingests more than 10 times its unfed body mass during its infrequent blood meals and subsequently eliminates its own body mass in urine every 20–30 min (Maddrell, 1991). During this postprandial diuresis, the Malpighian tubule (MT) cells, which produce the primary urine, exchange intracellular chloride content every 3 s (Ianowski et al., 2002) and secrete their own volume of isosmotic fluid every 15 s (Maddrell, 1991), as do tubule cells of another fluid feeder, the fruit fly (Dow et al., 1994).

The excretory system in insects consists of the MTs and the hindgut. The MTs secrete a primary urine that is near isosmotic to the haemolymph and contains KCl and/or NaCl as the predominant solutes, along with low molecular weight solutes that have either diffused into the lumen or been actively transported into it (O'Donnell, 2008). The tubules empty their contents into the gut at the midgut–hindgut junction. The primary urine is modified in a downstream segment of the tubule or, more commonly, in the hindgut, by the absorption of ions, water and useful metabolites such as amino acids and sugars, before excretion from the anus (Maddrell, 1981). The excreta contain elevated levels of nitrogenous waste and toxins and can be hypo-osmotic or hyperosmotic to haemolymph, depending on the extent of reabsorption of water and solutes (O'Donnell, 2008). The MTs thus provide the functional equivalent of the mammalian glomerulus, and the ileum and rectum of the hindgut perform the functions of the proximal and more distal segments of the nephron, respectively.

Fluid secretion by MTs is controlled by multiple factors which produce diuretic effects that can be quite dramatic. During postprandial diuresis in *Rhodnius*, the MT cells, which produce the primary urine, exchange intracellular chloride content every 3 s (Ianowski et al., 2002) and secrete their own volume of isosmotic fluid every 15 s (Maddrell, 1991), as do tubule cells of another fluid feeder, the fruit fly (Dow et al., 1994). There are five diuretic factors that stimulate fluid secretion by *Drosophila* tubules (Davies et al., 2013), and at least seven peptides and two amines that stimulate tubules of the adult hawk moth, *Manduca sexta* (Skaer et al., 2002). Although antidiuresis in insects historically was considered the result of increased absorption across the hindgut, there are several instances of antidiuretic peptides which slow secretion by the tubules. For example, cardioacceleratory peptide 2b (CAP2b)
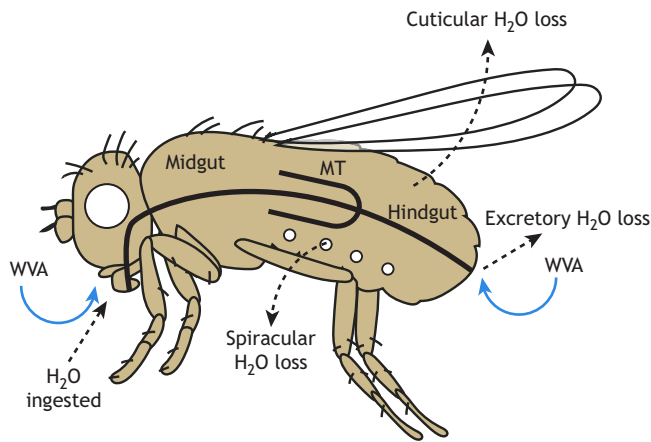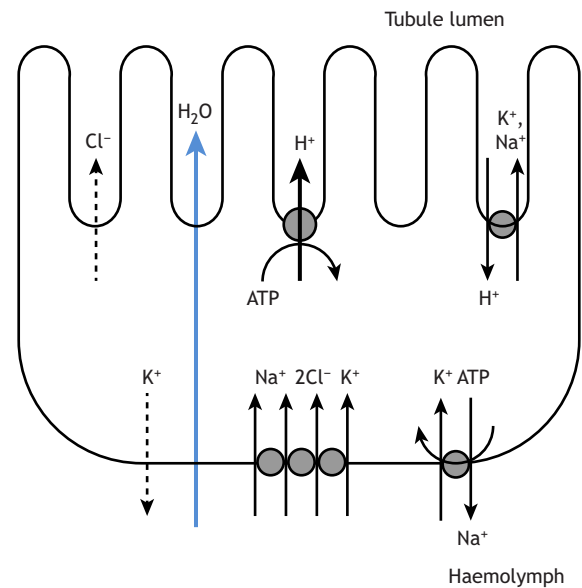
Fig. 1. Schematic figure showing pathways for gain and loss of water in insects. Ingested water is commonly transferred into the haemolymph across the midgut. Excretory losses are reduced by secretion of less fluid by the Malpighian tubules (MT) and/or enhanced recovery of solutes and water by the hindgut. Respiratory water losses may be reduced in some species by spiracular control mechanisms. Cuticular losses may be reduced by altering the composition of lipids in the layers covering the epicuticle. In some species, sites of reduced water activity on the mouthparts or within the hindgut may permit water vapour absorption (WVA; blue arrows). Although metabolic water is important in pupae, and in flying bees (Nicolson, 2009), it is generally less important in active insects (Benoit and Denlinger, 2010; Edney, 1977) and has not been included in the figure as a significant source of water.

Fig. 2. Model of a MT principal cell showing the two ATP-dependent ion pumps. The vacuolar type H^+-ATPase (V-ATPase) resides in the apical membrane and the Na^+/K^+-ATPase in the basolateral membrane. The V-ATPase can drive secretion of K^+ and Cl^- when extracellular fluids contain levels of Na^+ too low for effective operation of the Na^+/K^+-ATPase. When sufficient levels of both cations are present, operation of both basolateral and apical ATPases may aid Na^+ retention in the haemolymph and boost secretion of excess K^+. Water (blue arrows) moves in response to the osmotic gradients resulting from ion transport.

reduces fluid secretion rates in MTs of *R. prolixus* (Paluzzi, 2012; Quinlan et al., 1997). There are a number of possible reasons for the presence of so many diuretic and antidiuretic factors in insects. The locust diuretic peptide that is related to vertebrate corticotropin-releasing factor and a second diuretic peptide known as locustakinin work via different second messengers and differentially affect movements of Na^+ and K^+, thus altering the Na^+/K^+ ratio of the secreted fluid (Coast, 1995). Waste excretion by tubules or transport of organic molecules such as amino acids might, in principle, be altered by hormones. Secretion of potentially toxic organic cations by *Drosophila* tubules, for example, is increased by diuretic factors through direct enhancement of organic cation transporter activity (Bijelic and O'Donnell, 2005). The most intriguing possibility is that peptides and amines may act in concert with each other to produce physiologically distinct responses involving multiple tissues, not just the MTs. It has been suggested that there is a 'continuous broadcast of information in the form of a chemical language' in the extracellular fluid, and that this neurochemical fine-tuning ensures effective and efficient coordination of the functioning of multiple tissues (Skaer et al., 2002). The peptide leucokinin, for example, affects multiple tissues and plays roles not just in ion and water homeostasis but also in feeding, sleep, metabolism, memory, taste and nociception (Nässel and Wu, 2021). Biogenic amines such as serotonin, octopamine and tyramine also have widespread effects (Lange and Orchard, 2021).

The dominant energizer of ion transport in insect MTs is the vacuolar-type H^+-ATPase (V-ATPase) in the apical membrane (Fig. 2). H^+ is transported from cells to the tubule lumen, creating an electrochemical gradient favouring H^+ movement back into the cell that drives exchangers for K^+/H^+ or Na^+/H^+, and a favourable electrical gradient for the movement of Cl^- into the lumen (O'Donnell et al., 1996). In *Drosophila* tubules, the basolateral Na^+/K^+-ATPase plays a subordinate role, energizing the uptake of Na^+, K^+ and Cl^- through the $Na^+/K^+/2Cl^-$ cotransporter, and returning Na^+ to the haemolymph, thus minimizing the loss of Na^+

in the urine (Rodan et al., 2012). One possible advantage of the use of two ionomotive ATPases is that secretion of K^+-rich fluids can be accomplished in herbivorous species ingesting material that is K^+-rich but Na^+-poor, thus precluding effective operation of the Na^+/K^+-ATPase.

In vitro studies of MTs have been greatly simplified by the Ramsay assay, which allows secreted fluid to be collected from single tubules isolated in saline drops under paraffin oil (Ramsay, 1954). By contrast, measurements of fluid and ion transport by the hindgut have required use of more complex experimental preparations of everted sacs or flat sheets of tissue mounted in Ussing chambers and have been limited to larger species such as locusts. Classic studies by Phillips and his students have revealed mechanisms for absorption of KCl-rich, low Na^+ fluids from the locust hindgut (Phillips and Audsley, 1995; Phillips et al., 1996). The dominant process in the hindgut is active absorption of Cl^- across the apical membrane that is not coupled to, or driven secondarily by, fluxes of other ions. Absorption may involve a Cl^--ATPase that is stimulated through ion transport peptide (ITP) and chloride transport stimulating hormone (CTSH) utilizing cAMP as a second messenger. In contrast to MTs, the apical V-ATPase in the hindgut plays a subordinate role, transporting ions at <15% of the rate of Cl^- transport (Phillips et al., 1996).

The importance of the excretory system as an avenue for water loss depends very much upon the diet and water availability. Excretory water loss is a small proportion of the total (∼6%) in xeric and mesic *Drosophila* (Gibbs et al., 2003). By contrast, in feeding locusts, the gain of water in the food and loss in faeces are the most important avenues of gain and loss (Loveridge, 1975). Locusts fed grass with 85% water excrete frass with a water content of 77% and remain in positive water balance. When fed on bran with a water content of 5%, their frass water content drops to 36% but they still

lose water and thus will tend to restrict food intake (Edney, 1977; Loveridge, 1974). Recovery of water from the rectal lumen involves the creation of local osmotic gradients and ion recycling within specialized transporting epithelia called rectal pads in cockroaches and locusts (Wall and Oschman, 1970) and rectal papillae in flies (Gupta et al., 1980) (Fig. 3A). The pinnacle of water recovery from the hindgut is achieved in the cryptonephridial arrangement in larvae of beetles such as the mealworm, *Tenebrio molitor*: the distal ends of the MTs are applied to the haemolymph-facing surface of the rectal epithelium and enveloped in a perinephric membrane that is composed of as many as 40 circumferential layers of cells (Fig. 3B). Ions (Na^+, K^+, Cl^-) are transported into the tubule

Fig. 3. Arrangement of the MTs and gut in insects. (A) In many insects, water and ions are absorbed across the midgut into the haemolymph and then secreted into the lumen of the MTs (grey hatched arrows). Further reabsorption of water takes place downstream in the hindgut, often across specialized structures such as rectal pads. This arrangement allows recycling of water and ions by secretion into the tubules followed by reabsorption downstream (blue arrows). (B) Cryptonephridial arrangement in larvae of coleopteran beetles such as *Tenebrio molitor*. Cryptonephridial Malpighian tubules (cMTs) are present at the surface of the rectum and enveloped in a multilayered perinephric membrane (PNM). Ions (primarily K^+ and Cl^-) are secreted into the tubule lumen at specific sites which traverse the PNM. This arrangement limits water movement from the haemolymph to the tubule lumen in response to elevated osmotic pressures (OP) produced by KCl accumulation in the MT lumen. Water is recovered from the excreta or is condensed from atmospheric H_2O vapour. Water and ions are transferred into the haemolymph across the 'free' segments of the tubule downstream from the cryptonephridial complex and the ions are then recycled back into the cMT lumen (dashed line).
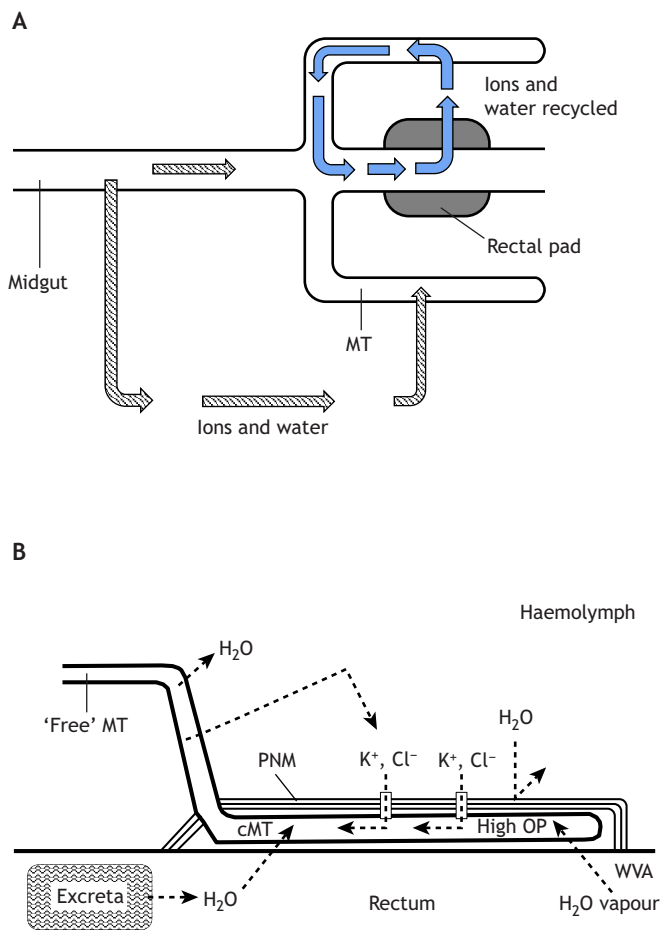
lumen at restricted sites, either the anterior region of the complex, where the perinephric membrane is thinner, or through specific leptophragma cells which interrupt the perinephric membrane and are present in tubules of some, but not all beetle species with a cryptonephridial system (O'Donnell and Machin, 1991; Saini, 1964). Irrespective of the site of ion entry, the perinephric membrane appears to function to limit osmotic water movements of water from the haemolymph into the tubule lumen, which accumulates high concentrations of K^+ (2700 mmol l^{-1}), Na^+ (400 mmol l^{-1}) and Cl^- (O'Donnell and Machin, 1991). The osmotic gradient created by elevated salt concentrations in the lumen of the cryptonephridial tubules is coupled instead both to recovery of water from the hindgut, resulting in production of powder-dry faecal pellets containing <17% water (Ramsay, 1964), and to the absorption of atmospheric water vapour, as discussed below.
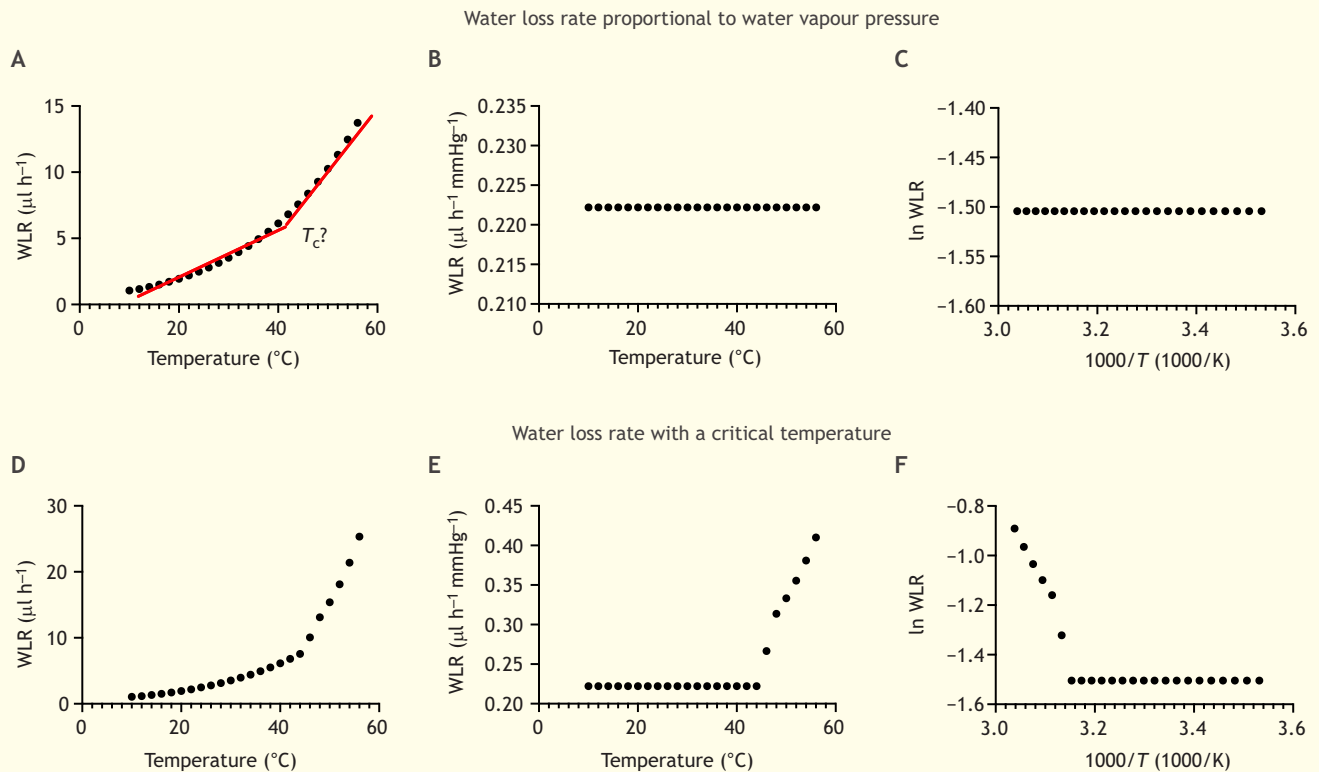
Cuticular water loss

Desiccation resistance of insects relies upon the thin (<1 μm) layer of waxy lipids in the epicuticle, the outermost layer of the cuticle. The lipids are secreted by specialized cells (oenocytes) associated with the epidermis and reach the epicuticle through pore canals running through the cuticular layers. There may be more than 100 lipid compounds in the epicuticle, including alcohols, esters, aldehydes, ketones, triacylglycerols, waxes, free fatty acids and long chain hydrocarbons of 21 to >40 carbon atoms. This last class of compounds are the predominant fraction and are termed cuticular hydrocarbons (CHCs) (Blomquist and Bagnères, 2010). CHCs are a mixture of straight or methyl-branched long-chain alkanes and alkenes. The physical properties of CHCs determine their roles in waterproofing and interspecific and intraspecific chemical signalling. Resistance to water loss generally increases modestly with CHC chain length, but is most affected by the degree of saturation. Methyl branched and unsaturated hydrocarbons do not pack as tightly as *n*-alkanes and thus have much lower melting points and are less effective as waterproofing agents relative to the highly hydrophobic linear *n*-alkanes.

Rates of water loss from insects are low at moderate temperatures and then increase dramatically above a specific critical temperature (T_c) in many species (Box 1). T_c is determined from an Arrhenius plot, in which the log of the water loss rate is plotted against the reciprocal of the temperature (in Kelvin). An abrupt shift to a steeper slope at higher temperatures coincides with the T_c, which, for some species, is very close to, or the same as, the melting point T_m (Box 1).

The increased water loss is attributed to the melting of the surface lipids (Gibbs, 1998, 2002). A complicating factor in considering melting is that the epicuticular layer is not homogeneous; there are spatial differences in thickness, composition and melting point (Hughes et al., 2011), and different layers of epicuticular lipids may exist in liquid or solid phases. Gibbs (2002) highlighted the importance of phase separation between melted and solid lipid fractions. Using 12 species of ants in which the CHC layer has been shown to be a solid–liquid mixture through the environmental temperature range, CHCs start melting between −60 and −45°C and melting is complete at 30–45°C (Menzel et al., 2017). When CHCs melt, trans–cis isomerizations of their carbon backbones create spaces, making it easier for water molecules to enter and diffuse through the lipid layer (Gibbs, 2011). Water thus diffuses through a disordered, high-entropy environment relative to densely packed lipids below the melting point. CHCs in many species are also important pheromones, and the mixture of lipid molecules synthesized

Box 1. Estimating critical temperature from plots of water loss rate versus temperature

Water loss rate proportional to water vapour pressure

Water loss rate with a critical temperature

Water loss rate (WLR) is shown for a hypothetical insect plotted in different ways. Discussions of critical temperature (T_c) in the literature have been complicated because some studies plot insect water loss versus temperature using linear scales. Because water vapour pressure increases exponentially with temperature, what is a continuous increase in slope on a linear plot can be misinterpreted as the intersection of two lines at 42°C, as shown by the red lines and 'T_c?' in A. There is no evidence for a critical temperature when WLR is corrected for vapour pressure deficit, for example at 50% relative humidity (as shown in B) or when the logarithms of the WLR data ($\mu l\ h^{-1}\ mmHg^{-1}$) are plotted in an Arrhenius plot (as shown in C) (Gibbs, 2002; Toolson, 1978, 1980). The Arrhenius equation for WLR can be expressed as a linear equation in the form:

$$\ln WLR = (E_a/R)(1/T) + \ln A, \tag{B1}$$

where E_a is a measure of the energy required for a water molecule to pass through the cuticle, R is the gas constant, T is the temperature (in K) and A is a constant. In Arrhenius plots of insect water loss data, the logarithm of WLR is plotted as a function of the reciprocal of the temperature A and the slope equals E_a/R. A true T_c is evident as an increase in WLR at a particular temperature, not just in linear plots (see D) but also when data are corrected for vapour pressure deficit (E) or plotted as an Arrhenius plot (F; where WLR is in $\mu l\ h^{-1}\ mmHg^{-1}$). The change in slope (E_a/R) of the Arrhenius plot in F at $1/T$=3.13 (corresponding to T=46°C) is indicative of a true temperature-induced alteration of cuticular permeability, such as would occur through a change in lipid fluidity (i.e. melting).

in a given species appears to represent a compromise between lower melting points, which favour dispersal for communication, and higher melting points, which minimize water loss.

Adaptive changes in water balance in populations from xeric versus mesic environments are often associated with changes in the composition of the cuticular water barrier. A reduction in water loss rate in desiccation-selected fruit flies is correlated not with an increase in the thickness of the waterproofing layer but with a change in composition, with an increase in the proportion of longer-chain CHCs with higher lipid T_m (Gibbs et al., 1997) and reduced proportions of unsaturated and methylated hydrocarbons (Stinziano et al., 2015). In ant species from different biogeographic regions, cuticular CHCs of species from wetter regions pack less effectively and have lower melting points, consistent with less effective waterproofing (Menzel et al., 2017). Changes in CHCs are also seen in response to changes in diet, humidity, mating, developmental stage or day length; although such changes are thought to influence

pheromonal communication primarily (Otte et al., 2018), there may be ancillary effects on waterproofing. Although the dominant role of CHCs in reducing cuticle water permeability is well accepted, it is also worth noting that melanization of the procuticle may increase resistance to water loss in some species (King and Sinclair, 2015; Rajpurohit et al., 2008).

Many studies have indicated the dynamic nature of the cuticular water barrier. Evidence for phenotypic plasticity of cuticular waterproofing was shown by desiccating flies for a few hours, sufficient to reduce water content by about 10%; this pre-treatment was correlated with reduced rates of cuticular water loss during subsequent desiccation of female flies (Bazinet et al., 2010). Increases in temperature heighten water stress, and changes in cuticle CHC composition indicate phenotypic plasticity in several species studied to date. Fruit flies exposed to higher temperatures produce higher concentrations of longer-chain CHCs; yet, surprisingly, this change is not correlated with lower rates of

water loss (Rajpurohit et al., 2021). Greater tolerance of desiccation of dry-adapted versus temperate populations in this study reflect not a reduction in water loss rate but perhaps an increase in body water content or tolerance of greater amounts of water loss. The dynamic nature of the water barrier is also apparent in studies of water loss rates in cockroaches. Mechanical contact in culture or handling results in cuticle damage and increased permeability that declines over a few days as the damage is repaired (Machin and Lampert, 1987). Early studies suggesting that cuticle permeability declines at lower levels of humidity (Loveridge, 1968; Machin et al., 1985) may have been a consequence of minor damage to the cuticle, as permeability of a damaged cuticle is humidity dependent, whereas permeability of repaired cuticle is constant over a wide range of relative humidity values (Machin and Lampert, 1987).

Respiratory water loss

Insect trachea are a ramifying system of air-filled tubes which allow the exchange of respiratory gases between tissues and the atmosphere (Nation, 2016). Air enters the trachea through openings in the exoskeleton called spiracles, which, in most species, can be opened or closed by contraction of associated muscles controlled by the central nervous system; some apterygote insects and larvae lack spiracular closing mechanisms (Dittrich and Wipfler, 2021).

Measured rates of water loss in insects, birds and mammals scale to oxygen uptake with slopes of 1 or close to 1, indicating that higher rates of gas exchange lead to higher water costs (Woods and Smith, 2010). Convection within the tracheal system can result from the indirect pumping of tracheal gases by autoventilation due to muscle contractions (of the body, heart or gut) that are not specific for gas exchange. However, the classic work of Weis-Fogh (1964) showed that even in insect flight muscle, the most metabolically active tissue known, simple diffusion through the tracheal system could account for 2–3 times the required rates of O_2 and CO_2 transport. Convective movements of gas in the tracheal system may thus have evolved for reasons other than increasing the supply of oxygen and eliminating carbon dioxide. Importantly, water loss can be minimized by switching from diffusive to convective exchange (Kestler, 1984). When gas exchange is convective, water vapour and oxygen flow at the same rate, whereas when gas exchange is diffusive, water is lost approximately 25% faster because of its higher rate of diffusion relative to oxygen. The ratio of the convective to diffusive rates of water loss per unit body mass scales with body mass$^{2/3}$, so the advantage of convective gas exchange will be greater the smaller the insect (Kestler, 1984).

Insect respiration is most easily monitored through measurement of CO_2 release, and three major respiratory patterns have been identified: discontinuous, cyclic or continuous gas exchange. Whereas some insects release CO_2 continuously, others show a cyclical pattern of low and high rates of release. Still others show a pattern known as discontinuous gas exchange (DGE), during which the spiracles are in one of three states: open, closed or fluttering. CO_2 is released in bursts when the spiracles open and at negligible rates when the spiracles are closed or fluttering. When the spiracles are closed, tissue metabolism reduces the partial pressure of O_2 in the trachea, and total gas pressure drops as the more soluble CO_2 produced by metabolism is buffered by the extracellular fluids. A sufficient drop in tracheal O_2 levels leads to transient openings of the spiracles referred to as spiracular flutter. Cyclic respiration is likely the ancestral pattern in insects and DGE is by no means ubiquitous; DGE has been recorded in species in 5 of 18 orders examined and appears to have evolved independently in each of the five (Marais et al., 2005). In non-diapausing insects (e.g.

Periplaneta americana, *Schistocerca gregaria*), the opening of the spiracles coincides with bouts of abdominal ventilation and ventilation is characteristic of both active and resting insects (Kestler, 1984).

For insects with spiracles, mathematical models suggest that insects can keep the spiracles closed a high percentage of the time during the flutter phase (and yet receive almost as much oxygen as if the spiracles were always open), provided the spiracles open and close rapidly (Lawley et al., 2020). As water loss is proportional to the proportion of time that the spiracles are open, a high frequency of flutter is consistent with both adequate oxygen uptake and low water loss, particularly for large insects (Lawley et al., 2020).

Diffusive loss of water vapour from the tracheal system is minimized during flutter if it is counterflow to the convective inflow of air. Water loss is clearly reduced during the flutter phase in cockroaches (Kestler, 1984), and meta-analyses provide support for water conservation during DGE in multiple other species (Oladipupo et al., 2022; White et al., 2007). However, in many species of insect, the flutter phase is absent, or only apparent in resting insects, or it disappears when temperature increases and water conservation would be more critical (Quinlan and Gibbs, 2006; Rourke, 2000).

Explanations for the adaptive value of DGE, other than water conservation, include protection from parasites (mites, bacteria, fungi) that may gain access through open spiracles, minimizing gas exchange as a means of reducing oxidative damage from O_2-derived free radicals (Bradley, 2007; Contreras et al., 2014) or allowing CO_2 to build up sufficiently so that it may efflux when the spiracles open for species living in subterranean environments with high ambient levels of CO_2 (Bradley, 2007; Lighton, 1996).

It is worth noting that respiratory losses, relative to transpiration across the cuticle, are in most instances a small fraction ($\sim$5–20%) of total water loss (Chown, 2002; Hadley, 1994; Quinlan and Gibbs, 2006). However, respiratory water loss and mechanisms for its reduction appear most important in xeric species, because respiratory water loss constitutes a larger proportion of total water loss in xeric species relative to mesic species of similar size and metabolic rate (Addo-Bediako et al., 2001).

Water vapour absorption

Convergent evolution of structures or processes in animals is generally interpreted as an indicator of the power of natural selection (Sackton and Clark, 2019). Powered flight, for example, has evolved 4 times (in insects, pterosaurs, birds and bats) because of the selective advantage conferred in terms of predator avoidance and the acquisition of food and mates (Pickrell, 2014). The independent evolution of arthropod water vapour absorption (WVA) at least 9 times, involving diverse mechanisms and multiple sites (coxal glands of mites, mouthparts of insects or ticks, rectum of insects, endopodal gills of isopods, body surface of collembolans), provides some indication of the importance of this process to terrestrial arthropods (see Table 1 for examples). For flightless insects, mites or ticks, whose dimensions may be far less than 1 mm, WVA may be particularly advantageous because their rates of locomotion may be insufficient relative to the water loss that occurs during travel between liquid sources of water. For small insects, rapid rates of diffusion in air mean that water may be more readily available in gaseous form than as a liquid. However, the technical challenges inherent in the measurement of atmospheric water uptake by small organisms, particularly by gravimetric methods, make it likely that many examples of WVA remain undiscovered. Application of less frequently applied isotopic (Wharton and Devine, 1968) or

Table 1. Selected examples of water vapour absorption (WVA) in insects and collembolans

Insect group/species	Site of WVA	Proposed mechanism	Reference
Desert cockroach (*Arenivaga investigata*)	Mouthparts (hypopharyngeal bladders)	Hydrophilic cuticle	O'Donnell, 1982
Tenebrionid beetles	Rectum	High KCl and NaCl concentrations in cryptonephridial complex	O'Donnell and Machin, 1991
Bark lice (Psocoptera) and bird lice (Mallophaga)	Mouthparts	Labial glands secrete fluid of reduced water activity	Rudolph, 1982b
Fleas (Siphonaptera)	Rectal sac	Secretion and reabsorption	Bernotat-Danielowski and Knülle, 1986
Silverfish, firebrats (Thysanura)	Anal sac	Electro-osmosis	Küppers et al., 1986
Collembola	Body surface	Accumulation of sugars and polyols in body fluids	Holmstrup et al., 2001

microcalorimetric (Wright and Westh, 2006) methods may reveal further novel mechanisms of this extraordinary process.

WVA in hygrophilic soil arthropods such as the common soil collembolan *Folsomia candida* is based on increasing body fluid osmolarity by accumulation of compatible osmolytes such as myoinositol and glucose, thus permitting passive osmotic water influx across the permeable body surface from near-saturated humidities (>99% RH) (Bayley and Holmstrup, 1999; Holmstrup et al., 2001). By contrast, WVA in insects that absorb water vapour in substantially subsaturated humidities requires both the creation of localized sites at which the concentration of freely diffusible water (i.e. water activity) is reduced within specialized tissues, typically the mouthparts or hindgut, and the minimization of water loss elsewhere through effective cuticular waterproofing. However, at decreasing body size, not only does water loss increase because surface area/volume ratio increases but also the thickness of the cuticle and its water barrier declines in proportion to the change in linear dimension (Kestler, 1984). WVA at a localized site may thus be an important means of offsetting water losses across the bulk of the body surface.

In the mealworm *T. molitor*, WVA is dependent upon the production of a highly concentrated solution of KCl and NaCl that is secreted into the lumen of the cryptonephridial MTs (Machin and O'Donnell, 1991; O'Donnell and Machin, 1991) (Fig. 3B). However, WVA by species such as the firebrat *Thermobia domestica* at 45% RH and the book lice and bark lice of the order Psocoptera at 60% RH cannot be based on KCl or NaCl as these salts become saturated at ~85% RH and ~75% RH, respectively. Other mechanisms of WVA that have been proposed include hydrophilic cuticle in the mouthparts of the desert cockroach and of ticks (Gaede and Knulle, 1997; O'Donnell, 1982), hygroscopic secretions of the labial glands in psocopterans and mallophagans (biting lice) (Rudolph, 1982a), and electro-osmosis in thysanurans (Küppers et al., 1986). It is worth emphasizing that energy may be used in different ways during WVA. Energy is required to produce highly concentrated solutions of inorganic ions in the cryptonephridial complex; WVA is then a passive consequence of the colligative lowering of water vapour pressure. By contrast, energy is required to remove water from hydrophilic mouthparts in ticks and the desert cockroach. Cyclical modification of the water affinity of the polyelectrolyte components of the cuticle through addition of low concentrations of inorganic salts has been proposed as a possible mechanism for the release of water from hydrophilic mouthparts (Gaede and Knulle, 1997; O'Donnell, 1982). Irrespective of the mechanism, classic studies revealed that although WVA may involve the movement of water against large thermodynamic gradients, the metabolic cost is quite low, typically only a few per cent of the basal metabolic rate (Edney, 1977).

Summary and future directions

Many aspects of insect physiology can be viewed as a response to the overarching need for water balance. No one feature is dominant; limiting respiratory, cuticular and respiratory water loss and supplementing water gain through intake of food and fluids or by WVA all contribute to overall water balance. It is important to note that although increases of water loss during activity may limit survival, the need to find food or mates and to avoid predation may necessitate such activity. Maintaining water balance is thus not always the preeminent stress on insects. Nonetheless, restriction of water loss across the body surface, the gut and the respiratory system undoubtedly enables their success as a terrestrial group, as does their ability to exploit foods and even the atmosphere as sources of water. The independent evolution of WVA mechanisms in at least six insect orders suggests that there may be other undiscovered examples, particularly in small insects, which have been difficult to study using gravimetric techniques. Further studies using classic radioisotopic methods (Wharton and Devine, 1968) or state of the art calorimetric (Wright and Westh, 2006) or hygrometric (Schilman et al., 2011) techniques may yield further examples of this extraordinary process. Future studies of insect water balance will also be aided by the application of transcriptomic methods that have helped elucidate mechanisms of insect epithelial ion transport (Kolosov and O'Donnell, 2022; Leader et al., 2018). RNAseq analysis of the MTs of mosquitoes, for example, highlights the different water stresses associated with the change from aquatic larvae to terrestrial adults (Li et al., 2017). Further, such studies may help us understand the changes associated with adaptation to drier habitats, for example. Given the importance of insects in ecology, agriculture and human health (Scudder, 2017), the most important area for future research will be to determine the impact of anthropogenic climate change on insect populations (Ma et al., 2021; Pincebourde and Woods, 2020). Temperature and humidity changes alter both water loss rates and food water content, thus affecting insect survivability. Stresses on water balance mechanisms in a warming climate, therefore, may contribute to changes in insect populations. Resistance to water loss can be increased by increasing the amount of water in the body, reducing rates of water loss or tolerating a reduction in body water. Reduction of the water loss rate appears to play the dominant role in desert species of *Drosophila* (Gibbs et al., 2003), and comparable studies of insect species in other orders will be important in predicting the impact of climate change on insect numbers and biodiversity.

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