Classification

Biological Sciences: Ecology
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Title

Super-hydrophobic diving flies (*Ephydra hians*) and the hypersaline waters of Mono Lake

Short Title

Super-hydrophobic diving flies of Mono Lake

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Abstract.

The remarkable alkali fly, *Ephydra hians*, deliberately crawls into the alkaline waters of Mono Lake to feed and lay eggs. These diving flies are protected by an air bubble that forms around their super-hydrophobic cuticle upon entering the lake. To study the physical mechanisms underlying this process, we measured the work required for flies to enter and leave various aqueous solutions. Our measurements show that it is more difficult for the flies to escape from Mono Lake water than fresh water, due to the high concentration of Na_2CO_3 which causes water to penetrate and thus wet their setose cuticle. Other less kosmotropic salts do not have this effect, suggesting that the phenomenon is governed by Hofmeister effects as well as specific interactions between ion pairs. These effects likely create a small negative charge at the air-water interface, generating an electric double layer that facilitates wetting. Compared to six other species of flies, alkali flies are better able to resist wetting in a 0.5M Na_2CO_3 solution. This trait arises from a combination of factors including a denser layer of setae on their cuticle and the prevalence of smaller cuticular hydrocarbons compared to other species. Although superbly adapted to resisting wetting, alkali flies are vulnerable to getting stuck in natural and artificial oils, including dimethicone, a common ingredient in sunscreen and other cosmetics. Mono Lake's alkali flies are a compelling example of how the evolution of pico-scale physical and chemical changes can allow an animal to occupy an entirely new ecological niche.

Significance.

Super-hydrophobic surfaces have been of key academic and commercial interest since the discovery of the so-called “lotus effect” in 1977. The effect of different ions on complex super-hydrophobic biological systems, however, has received little attention. By bringing together ecology, biomechanics, physics, and chemistry, our study provides new insight into the ion-specific effects of wetting in the presence of sodium carbonate, and its large-scale consequences. By comparing the surface structure and chemistry of the alkali fly—an important food source for migrating birds—to other species, we show that their uniquely hydrophobic properties arise from very small physical and chemical changes, thereby connecting pico-scale physics with globally important ecological impacts.

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Introduction.

In late summer, the shores of Mono Lake, California, are bustling with small flies, *Ephydra hians*, which crawl under water to feed and lay eggs (Fig. 1A). Their unusual behavior was eloquently described by Mark Twain during his travels to Mono Lake (1),

> *"You can hold them under water as long as you please--they do not mind it--they are only proud of it. When you let them go, they pop up to the surface as dry as a patent office report, and walk off as unconcernedly as if they had been educated especially with a view to affording instructive entertainment to man in that particular way."*

Although Twain's observations are over 150 years old, we still do not understand the chemistry and physics underlying the ability of these flies to resist wetting as they descend below the water surface. Alkali flies are found on nearly every continent, and fulfill an important ecological role by transforming the physically harsh environments of alkaline lake shorelines, including the Great Salt Lake in Utah and Albert Lake in Oregon, into important wildlife habitats (2). Aside from the flies, only algae, bacteria, and brine shrimp tolerate Mono Lake's water, which is three times saltier than the Pacific Ocean, and strongly alkaline (pH=10) due to the presence of sodium bicarbonate and carbonate. For the past 60,000 years, Mono Lake has had no outlet (3), driving a steady increase in the concentration of mineral salts through a yearly evaporation of 45 inches (4). Calcium from natural springs underneath the lake's surface reacts with the carbonate rich water, precipitating calcium carbonate in the form of underwater towers called tufa. Alkali flies crawl underwater by climbing down the surface of the tufa, which have become exposed due to falling lake levels (Fig. 1B, Movies S1-2).

For the alkali fly, staying dry is paramount to their survival; if they do get wet in Mono Lake, a thin film of minerals dries on their cuticle, which makes them more likely to be wetted in subsequent encounters with the water. Like most insects, the flies are covered in a waxy cuticle festooned with tiny hairs (setae). As in water striders (5), these hydrophobic hairs trap a layer of air, so that as a fly crawls into the water an air bubble forms around its body and wings. The bubble protects the flies from the salts and alkaline compounds present in the lake, and also serves as an external lung (6), allowing flies to spend up to 15 min underwater crawling to depths of 4-8 m (7). Once finished feeding or laying eggs, the flies either crawl to the surface or let go of the substratum and float up. As noted by Twain (1), the bubble pops when it hits the air-water interface, depositing its inhabitant safe and dry on the water's surface (Fig. 1C, Movies S3-4). In this paper, we describe the physical and chemical properties that make the alkali flies uniquely able to form these protective bubbles in Mono Lake's dense and alkaline waters.

As a preamble to our measurements, we briefly review the physics of solid-liquid interactions. On smooth surfaces, the shape of an adhering liquid droplet may be described by the contact angle, with larger contact angles corresponding to less wettable surfaces (Fig. 1D). The contact angle for a smooth piece of waxy insect cuticle is typically 100-120°, similar to paraffin wax, and close to the theoretical maximum (8, 9). On rough surfaces – like that of an alkali fly, a liquid drop can exist in two different states. In the Cassie-Baxter state, air pockets fill the space between roughness elements (10), resulting in 'super-hydrophobicity' (a.k.a. the 'lotus-effect' (11)). In the Wenzel state, the liquid replaces the air pockets (12), resulting in a fully wetted surface. Whether a liquid-surface interface exists in the Cassie-Baxter or Wenzel state is a complex function of the surface's physical and chemical structure, the chemistry of the solution, and the interactions between the surface and the liquid. In the case of an alkali fly crawling into water, the combination of hydrophobic wax and setose surface favors the Cassie-Baxter state, rendering the flies super-hydrophobic (6, 9, 13), with contact angles approaching 180°. Other insects that make air-water transitions, including spiders and beetles, sport small patches with super-hydrophobic properties used for plastron respiration (6).

Results

To investigate which chemical and physical properties of the flies and Mono Lake water (MLW) influence the formation of the air bubble that protects them from the mineral-rich water, we built an optical force sensor (Fig. 2A), which we used in a manner similar to the Wilhelmy Balance Method (14) to measure the forces required for flies to enter and exit different solutions. We glued the flies to a tungsten beam (0.26 mm diameter), and slowly submerged them using a linear motor (speed 0.3 mm s^{-1}). The average peak force required for flies to enter MLW was approximately 1 mN, roughly 18x the body weight of the 5.5 mg flies (Fig. 2B, S1A). The force required to enter the water varied with body orientation, with a minimum at a vertical, headfirst orientation (Fig. S1B). This corresponds with our observations of flies at Mono Lake, which tend to enter the water by crawling down 45°-90° surfaces.

In pure water, the work required to submerge the fly is largely recovered when it is pulled out of the water—the surface tension of the bubble stores the potential energy much like a spring. Thus, we use the term *recovered work* (Fig. 2C) as a measure of how easy it is for the flies to escape the water. A positive value indicates a net upward force that pushes the fly out of the water, whereas a negative value indicates that the fly is partially wetted and trapped by surface tension at the air-water interface. With increasing concentration of MLW from 0% to 200%, we found that the recovered work decreases, despite the increase in solution density (Fig. 2D).

MLW contains a number of salts including NaCl, Na_2SO_4, and K_2SO_4, as well as the alkali components sodium bicarbonate and boric acid (15). To determine which of these components most influences the recovered work, we made two solutions, each containing double the natural concentration of either the salts or alkali compounds. Sodium bicarbonate is an alkali buffer in which the ratios of CO_3^{2-}, HCO_3^{1-}, and H_2CO_3 are coupled to pH according to the Henderson-Hasselbalch equation. To achieve a pH equal to that of MLW (pH=10), we used a molar ratio of $NaHCO_3$ to Na_2CO_3 of 0.8. Compared to MLW, recovered work was higher for the salt solution, whereas it was significantly lower for the alkali solution (Fig. 2E), implying that the alkali compounds make it more difficult for the flies to escape the water. Next, we tested three sodium bicarbonate buffer solutions ranging in pH from 8.5 to 11.6, and found that high pH significantly decreased recovered work (Fig. 2F-G).

Our results with the bicarbonate buffer solution suggest that the naturally high pH of the lake makes it more difficult for the flies to escape the surface. To directly test this hypothesis, we neutralized MLW with HCl, bringing the pH down to 7, which should slightly shift the carbonate balance towards HCO_3^{1-}. Compared to natural MLW, this neutralized solution did not significantly increase the recovered work (Fig. 2H). Testing a different alkali solution (5 mM NaOH) at the same pH as the highest pH sodium carbonate buffer (11.6), further confirmed that pH alone does not determine the amount of recovered work (Fig. 2I). We next tested the possibility that the concentration of Na_2CO_3 played a critical role by measuring the recovered work in three solutions: 0.5M Na_2CO_3 (pH 11.6), 0.15M Na_2CO_3 (pH 11.6), and 0.5M Na_2CO_3 neutralized with HCl to pH 7. We found the recovered work was lowest for the 0.5M Na_2CO_3 solution (Fig. 2J), which together with the buffer experiments from Fig. 2F-G, indicate that a high concentration of Na_2CO_3 makes it more difficult for the flies to escape the water surface.

To test whether the alkali flies' possess a unique adaptation to live in Na_2CO_3 rich waters, we compared their ability to recover work in distilled water, MLW, and a 0.5M Na_2CO_3 solution, to that of six dipteran species, including two other members of the Ephydridae (shore flies), two coastal kelp flies (adapted to living under constant salty ocean spray), and two cosmopolitan drosophilids. (Fig. 3A). All species were similar to alkali flies in that the work recovered from distilled water scaled with body length (Fig. 3B), which is expected because surface tension forces on a floating object are a function of contact perimeter (16). However, work recovered from MLW and the Na_2CO_3 solution was significantly lower in the other species. Of all species tested, only the alkali fly was pushed out of the Na_2CO_3 solution, suggesting that they have unique adaptations that render them super-hydrophobic in the presence of Na_2CO_3.

To investigate the physical differences between flies, we imaged samples of each species with a scanning electron microscope (SEM). The alkali flies possess a denser mat of setae on their bodies and legs (Fig. 3C-D and Fig. S2), and lack obvious pulvilli between their tarsal claws (Fig. 3E). In observing the other fly species in our plunging experiments, it is clear that the presence or absence of pulvilli does not play a critical role. In trials in which recovered work is negative, the entire body was wetted, not just the tarsi (Fig. 3F, Movies S8-9). To quantify the hairiness of each species, we used image processing to calculate the number of hair-crossings per μm for SEM image transects perpendicular to the mean hair orientation (see Supplemental Materials). Based on these metrics, the alkali flies are generally hairier than the other species: wings (+34%), thorax (+44%), abdomen (+47%), tarsi (+17%), and overall average (+36%). However, they are only 15% hairier than *Fucellia rufitibia* (a kelp fly). To summarize, body length explains 57% of the variance in recovered work in pure water across all seven species (65% if alkali flies are excluded), but only 0.009% for a 0.5M Na_2CO_3 solution (but 57% if alkali flies are excluded). After removing the body-size trend from the data for the Na_2CO_3 solution, a positive correlation between hairiness and recovered work explains 58% of the remaining variance.

To determine whether the flies' cuticular hydrocarbons might act in combination with the setae to prevent wetting, we briefly rinsed flies in hexane and measured the recovered work in MLW and distilled water. We found that hexane removed compounds that are important for the fly to stay dry in MLW, but not pure water (Fig. 4A). Next, we analyzed the cuticular hydrocarbons of all seven species with GCMS (see Methods for details). The cuticular hydrocarbon profile of alkali flies is dominated by straight-chain alkanes (pentacosane [C25] and heptacosane [C27]) (Fig. 4B). The two other members of Ephydridae were similar, whereas the two drosophilids had a higher abundance of larger alkenes, dienes, and methylated even-numbered hydrocarbons. The kelp flies exhibited very different profiles dominated by tetra-methylated C30, and C21 (Fig. 4C).

To verify the reproducibility of our results, we developed a simplified assay to test the effects of different solutions on flies' ability to escape from a liquid-air interface. We used the easily reared species, *Drosophila virilis*, for these experiments because they require large numbers and we did not wish to sacrifice so many wild caught *Ephydra*. In these trials, we briefly anesthetized 20 flies with CO_2 and sprinkled them onto a 44 cm^2 surface of nine solutions, each at five concentrations. Sodium carbonate was the most detrimental to the flies' ability to escape compared to other salts (in particular at intermediate concentrations), even when compared with solutions of pH>13, those containing divalent anions, or K_2CO_3 (Fig. 5). The small effect of K_2CO_3 compared with Na_2CO_3 suggests that the enhanced wetting caused by Na_2CO_3 is not solely a property of the carbonate ion, but also its interaction with the sodium ions.

Our working hypothesis is that the presence of Na_2CO_3 biases the liquid-cuticle interaction to favor Cassie-Baxter-to-Wenzel state transitions. To try to observe this phenomenon more directly, we developed another preparation that makes use of the long fine hairs found on the trailing edge of many insect wings. Wetting, or its absence, is easy to visualize when this 2-dimensional array of hairs is placed in contact with a water drop. For these experiments, we chose the common house fly, *Musca domestica,* due to its large size and availability. We directly filmed the interaction between the wings when repeatedly pressed against drops of either pure water or a 0.5M Na_2CO_3 solution. In only one of nine wings did a tiny droplet of pure water stick to the wing (Movie S8). In the case of 0.5M Na_2CO_3, however, four of the nine wings showed large drops adhered to the wing, and one with a tiny droplet (Movie S9). The influence of Na_2CO_3 might act directly on the cuticle surface, or it might involve a more complex mechanisms involving geometry of the fine hairs and the spaces between them. To test between these possibilities, we needed a sufficiently large piece of flat chitin on which we could accurately measure contact angles. Because insects are too small and setose, we made clean, flat preparations from shrimp exoskeletons for these tests. We measured no difference in the contact angle for water and 0.5M Na_2CO_3 (water: 81±14° (mean±std), carbonate: 76±15°; N=18 each; T-test: p=0.31, t-stat=-1; 2 μL static sessile drop technique, see Methods). Although shrimp cuticle lacks the hydrocarbons found on insects, chitin and hydrocarbons have roughly similar surface free energies (17, 18), and thus Young's equation predicts that they will have similar contact angles as well (14, 19). These results suggest that Na_2CO_3 acts to favor the Wenzel state by a mechanism involving the fine air pockets between hairs.

In the course of our field-work, we frequently observed large numbers of flies that were wetted and drowned on the surface of Mono Lake. We hypothesized that such events were due to oils from decaying organic matter that made it more difficult for flies to escape the water. When dropped onto MLW coated in a thin film of fish oil (20 μL over 44 cm^2), alkali flies immediately became trapped on the surface like a bird in an oil spill (Fig. S3A-B, Movies S10-11). In addition, while collecting water for our experiments, we occasionally noticed a thin film of sunscreen coming off of our skin and considered whether this might also deleteriously influence hydrophobicity. We measured the forces on alkali flies dipped into untreated MLW, and MLW used to rinse our hands 5 and 15 minutes after applying sunscreen. The sunscreen indeed had a catastrophic effect on the flies' ability to stay dry (Fig. 6A-C). To examine this effect in more quantitative detail, we applied measured amounts of sunscreen to wooden applicator sticks, and stirred them in pure water for 1 min. After briefly anesthetizing them with CO_2, we dunked the flies underwater and scored each fly after 15 min for either having flown away or gotten stuck. Amounts of 8 to 40 mg (applied to the wooden sticks) of the Neutrogena Ultrasheer SPF 50 Sport sunscreen raised the fraction of trapped flies from 50% to 100% (Fig. 6D). We then tested 20 mg applications of 6 different brands, and found that the three which had a deleterious effect all contained dimethicone (Fig. 6E), which was absent from the three neutral brands (Fig. S3C). We repeated the dunk assay with pure water after applying a surface film of 0, 2, or 10 μL of dimethicone (viscosity 5 cSt, Sigma Aldrich). As little as 45 nL of dimethicone per cm^2 of water was enough to trap 50% of the flies (Fig. 6F). Other artificial polymers such as trimethylsiloxysilicate and vp-hexadecenecopolymer are likely also problematic (Fig. S3C).

Discussion

Through a series of experiments with solutions varying in salinity, pH, and charge density, we showed that a high concentration of Na_2CO_3 makes it more difficult for alkali flies to escape from the surface of water by facilitating the penetration of water into the air pockets between individual hairs. The effect of Na_2CO_3 is surprising, considering that, like most salts, Na_2CO_3 increases the surface tension (+2% for 1M solution) (20), as well as the density of water. Both effects should theoretically increase the recovered work by making large bubbles more stable, and providing a larger buoyancy force. The fact that other salts, including K_2CO_3, have a significantly smaller effect demonstrates that this phenomenon involves interactions of specific ion pairs, and not with Debye-Hückel or Derjaguin-Landau-Verwey-Overbeek models. Instead, we offer a model based on the Hofmeister series to explain this phenomenon, which we term *ion-facilitated wetting*.

In 1888, Franz Hofmeister (21) discovered that certain ions are more likely to precipitate proteins out of egg white. The same order of compounds—now known as the Hofmeister Series—explains a wide range of phenomenon including solubility and surface tension (22). The order of anions is:

$$ClO_4^- < I^- < Cl^- < F^- < OH^- < PO_4^{3-} < SO_4^{2-} < CO_3^{2-}.$$

Ions to the right of Cl^- (kosmotropes) attract large hydration shells that structure the surrounding water, thereby increasing surface tension and driving ions away from the air-water interface. Ions to the left of Cl^- (chaotropes) have the opposite effect; they tend to accumulate at the surface of an air-water interface (23). Cations are arranged in following order:

$$NH_4^+ < K^+ < Na^+ < Li^+ < Mg^{2+} < Ca^{2+}.$$

Again, ions on the right have larger hydration shells, although anions generally have a more pronounced effect than cations. The underlying principles that give rise to the Hofmeister series are not well understood; however, the sequence is generally correlated with the ratio of ionic charge to ionic radii (Fig. 7A) (values from (24)).

Our result that Na_2CO_3, but not K_2CO_3, has a strong effect on wetting suggests that ion-facilitated wetting is dependent on the precise combination of cations and anions. Typically, Hofmeister effects of anions and cations are considered to be independent of one another (25), however, some phenomena are known to depend on ion specific pairs, such as the inhibition of bubble coalescence (26, 27). The physical basis of ion-facilitated wetting and bubble coalescence are likely related, because both have macro-scale consequences similar to those caused by surfactants (which increase wetting and decrease bubble coalescence), yet they operate through a completely different mechanism. However, our results cannot be explained by the current models of bubble coalescence. We propose that the relative distance of the cations and anions from the air-water interface plays a crucial role. Figure 7B illustrates how, as a rough approximation, CO_3^{2-} is on average situated 0.04 nm closer to the surface than Na^+ in Na_2CO_3 solution. This suggests that the carbonate ions will have a larger influence on the surface characteristics in the presence of Na^+, giving the air-water interface a slight negative charge. Taking this relative distance as well as the ion charge density into account explains 77% of the variance in the correlation with the likelihood of flies becoming trapped at the surface (Fig. 7C). In contrast, in a K_2CO_3 solution the ions are nearly equidistant from the surface. According to our theory, $CaCO_3$, $MgCO_3$, and Li_2CO_3 would have even stronger effects on wetting, as the hydration shells of these cations are even larger. However, these salts are only soluble at exceptionally low concentrations (0.1 mM to 0.17 M), not the ~0.5M concentrations that are necessary, which makes Na_2CO_3 the most potent compound for ion-facilitated wetting.

The physical mechanism by which the slight negative charge at the air-water interface might increase the likelihood of wetting is not immediately clear. One possible explanation involves electrostatic attraction between the flies' surface and the negatively charged fluid layer. Recent research has shown that Cassie-Baxter-to-Wenzel state transitions are more likely to occur in the presence of an applied voltage, which causes the formation of an electric double layer (28–30). The electric double layer increases the attraction between the interfacial water and individual roughness elements on the surface, thereby pulling the solution into the gaps and facilitating the transition to the wetted state. In experiments in which the distance between roughness elements was 4 μm (alkali flies' hairs are 3.2 μm apart), a voltage of 22 V was required to cause wetting (30). To relate these experiments with our results, we performed a rough calculation to determine the molar concentration of Na_2CO_3 needed to generate a 22 V potential between the water surface and the flies' cuticle (see Supplemental Materials). Our model suggests that a molarity of ~0.15 M of Na_2CO_3 is necessary to induce wetting, which is within a factor of 4 of the molarity we observed as necessary in our experiments, suggesting that this is a plausible mechanism warranting further study. A more thorough investigation would require detailed simulations of molecular dynamics that are beyond the scope of this paper.

Our theory also explains the role of the cuticular hydrocarbons in preventing wetting. The cuticle underneath the hydrocarbon layer is largely composed of chitin, which is slightly polar (static dielectric permittivity = 15 (31)). Thus, the non-polar hydrocarbon layer (static dielectric permittivity = ~2 (32)) helps to insulate the chitin surface from the electric double layer, reducing the likelihood of wetting. This theory is consistent with our finding that cuticular hydrocarbons do not influence wetting in pure water, as there would be no electric double layer.

Compared to the six other species we investigated, *Ephyrda hians* were the only species that resisted wetting in the presence of Na_2CO_3, an adaptation that allows them to occupy a rare but ecologically important niche. Remarkably, the trait that allows them to forage and lay eggs in such an extreme aquatic environment arises from just a few minor changes in physical and chemical properties. These adaptations likely evolved over time in response to the slowly increasing concentration of mineral salts (such as Na_2CO_3) in alkaline lakes across the world. In recent times, the selective pressures on the alkali flies at Mono Lake have become even stronger. Between 1941 and 1982, the concentration of mineral salts in the lake doubled as a result of Los Angeles' policy of diverting water from the Eastern Sierra. Our experiments, however, suggest that this increase in ion concentration has had only a small influence on the flies' ability to dive and resurface in the lake. By comparison, the increasing salinity has had a much larger detrimental effect on the flies' larvae (33).

The most important adaptation that made the niche of underwater feeding available to the alkali fly, however, was not a physical or chemical one. Rather, it was the behavioral urge to crawl under water and forage in the first place. We suspect that their ancestors evolved this unusual behavior in lean times, when surface food was a limiting resource but underwater algae were abundant. In addition, selection against underwater foraging presumably decreased in alkaline lakes, because the caustic chemistry makes them uninhabitable for fish.

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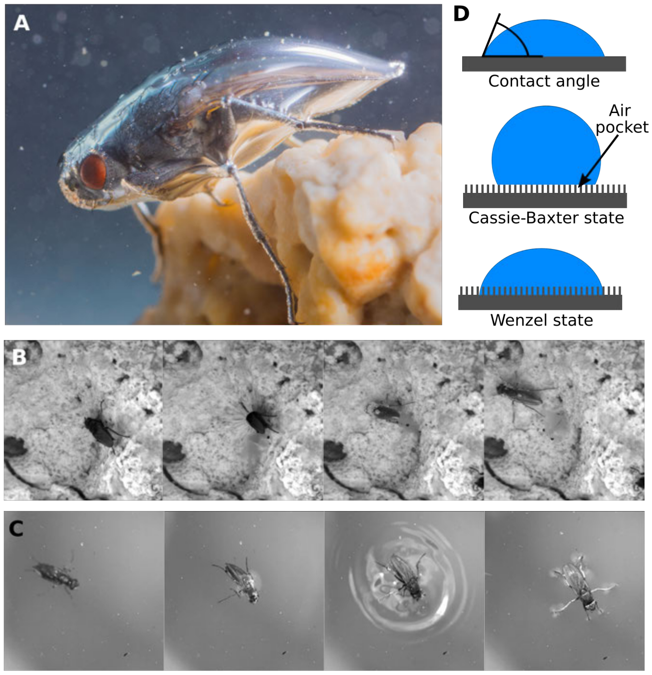


Figure 1. Mono Lake's alkali flies must exert up to 18 times their body weight to crawl under water to feed and lay eggs. (A) Close up of an alkali fly under water. **(B)** Image sequence of a fly crawling into the water (Movie S1-2). **(C)** Image sequence of a fly floating upwards to the surface inside its air bubble (Movie S3-4). **(D)** Illustrations of a water droplet on smooth and rough surfaces.

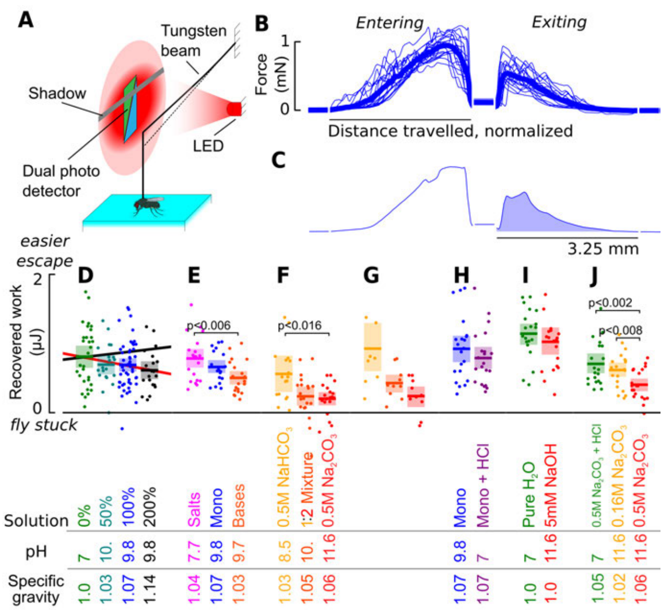


Figure 2. High concentrations of sodium carbonate make it more difficult for flies to escape from MLW. (A) Diagram of optical force sensor. Forces on the fly deflect the beam, shifting the shadow cast by an LED, which is detected by a photo detector. **(B)** Force vs distance travelled (normalized to fly height) for 20 CO_2 anesthetized flies dipped into MLW (bold: mean). Positive values correspond to upward forces (see Movie S5). **(C)** One example trace from A. Shading indicates the amount of work done on the fly as it exits the water, which we term recovered work. **(D)** Recovered work for different concentrations of MLW, ranging from pure de-ionized water to double strength MLW (produced via evaporation); 0% (N=30), 50% (N=20), 100% (N=50), 200% (N=20). Black line: expected recovered work based on the measurements in pure water and the increase in solution density. Red line: data regression ($p=0.003$; $r^2=0.08$). **(E)** Recovered work for salt or alkali solutions. Salt solution (double concentration of the salts in Mono Lake): 1.3M NaCl; 0.2M Na_2SO_4; 0.04M K_2SO_4; 1.8mM K_3PO_4. Alkali solution (double concentration of the alkali compounds in Mono Lake): 0.35M $NaHCO_3$; 0.44M Na_2CO_3; 0.09M Boric Acid. **(F)** Recovered work for a 0.5M $NaHCO_3$ buffer solution at three different pH values. **(G)** Same as F, showing only the subset of 10 flies that were dipped in the order of increasing pH. **(H)** Recovered work for standard MLW, and MLW neutralized to pH 7 with HCl. **(I)** Recovered work for pure water and 5mM NaOH, at the same pH as the carbonate buffer in E-F. **(J)** Recovered work for HCl-neutralized 0.5M carbonate buffer, 0.16M carbonate buffer, and 0.5 carbonate buffer. In this experiment, all flies were dipped in the order from left to right. **(D-J)** Shading indicates bootstrapped 95% confidence intervals. Non-overlapping shading generally corresponds to statistical significance of $p<0.02$. Resampling test statistics are given for cases in which differences are not obvious. D-G and H-J come from two separate collections, which may explain the slightly higher water repellency in H-J than would be expected based on D. The order of solutions into which the flies were dipped was alternated for each set of experiments unless otherwise noted. E-J, N=20. The Bond number for flies in the 0.5M Na_2CO_3 solution is 0.46, indicating that surface tension forces are dominant (see Supplement).

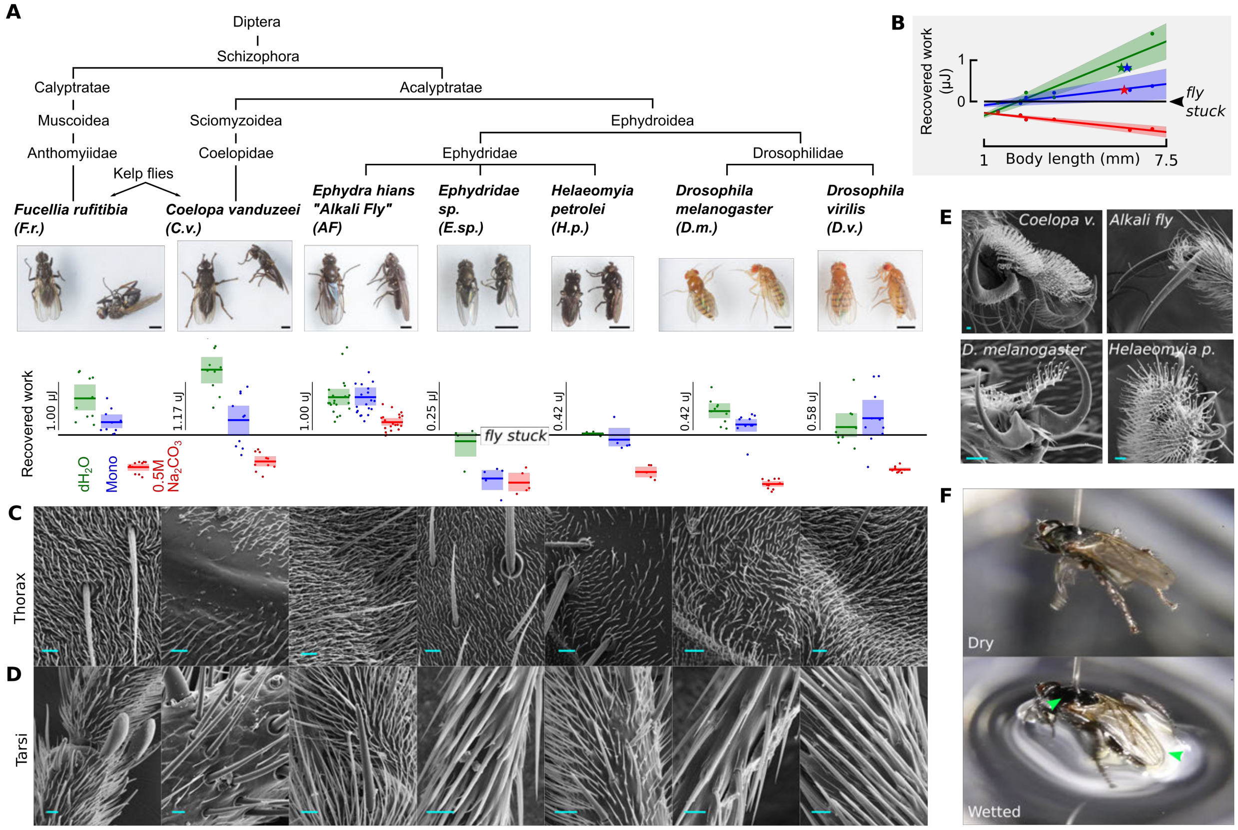
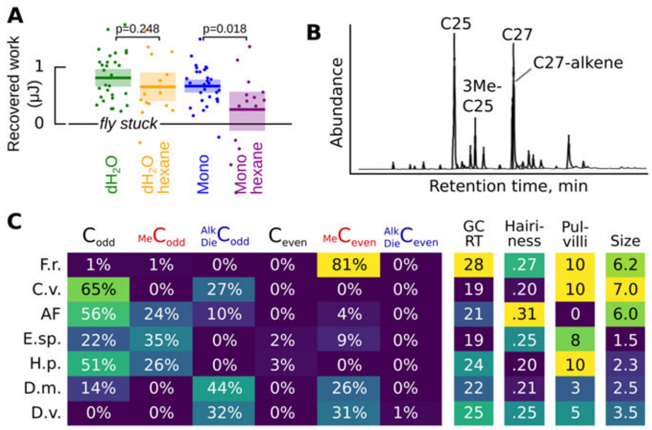


Figure 3. Mono Lake's alkali flies are uniquely adapted to withstand wetting in alkali water. **(A)** For each of 7 species, we measured the recovered work for pure water, MLW, and a 0.5 M Na_2CO_3 solution. Only the alkali fly were actively propelled out of the carbonate solution; all other species were stuck at the surface. See Movies S6-7. Scale bars are 1 mm. N=10 for Fr, Cv, Dm, Dv; N=20 for Eh; N=5 for Hp, Esp. **(B)** Correlation between body length and recovered work from panel A. Alkali flies are indicated by a star, and were omitted from the regressions. Shading indicates 95% confidence interval for the slope of the regression. **(C-D)** SEM images of the thorax and tarsi for each fly species. Scale bars are 10 µm. **(E)** The alkali fly is unique among the species examined in its lack of pulvilli. SEMs of three other representative species are shown (see also Fig. S2). Scale bars are 10 µm. **(F)** Cv before and after being dipped into 0.5M Na_2CO_3.



	C_{odd}	$^{Me}C_{odd}$	$^{Alk}_{Die}C_{odd}$	C_{even}	$^{Me}C_{even}$	$^{Alk}_{Die}C_{even}$	GC RT	Hairiness	Pulvilli	Size
F.r.	1%	1%	0%	0%	81%	0%	28	.27	10	6.2
C.v.	65%	0%	27%	0%	0%	0%	19	.20	10	7.0
AF	56%	24%	10%	0%	4%	0%	21	.31	0	6.0
E.sp.	22%	35%	0%	2%	9%	0%	19	.25	8	1.5
H.p.	51%	26%	0%	3%	0%	0%	24	.20	10	2.3
D.m.	14%	0%	44%	0%	26%	0%	22	.21	3	2.5
D.v.	0%	0%	32%	0%	31%	1%	25	.25	5	3.5

Figure 4. Increased hairiness and a coating of C25 cuticular hydrocarbons help the alkali flies resist wetting in MLW. (A) Recovered work for pure water and MLW, before and after alkali flies were rinsed in hexane for three one-second sessions. Flies were dipped in one of two orders: (1) deionized water, MLW, hexane treatment and a final dip in MLW (N=10); (2) MLW, deionized water, hexane treatment and a final dip in deionized water (N=10). **(B)** GCMS analysis of hexane extracted cuticular hydrocarbons of the alkali fly. **(C)** Relative abundance of hydrocarbons found by GCMS in hexane extracts of each species; average of the retention times (in min) for all of the GCMS peaks (weighted by relative abundance); mean number of hairs per µm (averaged across thorax, abdomen, wings, and tarsi); approximate body length of the species, in mm; and subjective relative size of the pulvilli. Codd: odd-length straight-chain hydrocarbons, e.g. C25, C27. MeCodd: methylated odd-length carbon chains, e.g. 3Me-C25. Alk/Die Codd: odd-length carbon chain alkenes and dienes. Ceven: even-length straight-chain hydrocarbons, e.g. C26, C28.

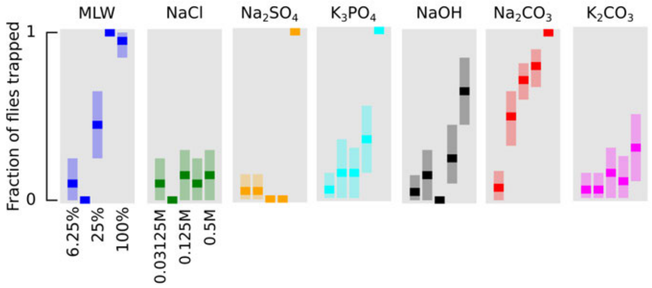


Figure 5. Sodium carbonate is more detrimental to flies' ability to escape compared to other salts. For each concentration of each chemical tested, we briefly CO_2 anesthetized 20 *Drosophila virilis* and sprinkled them onto a 400 mL jar (44 cm^2 surface area). 15 minutes later we scored each fly for having escaped (0) or being trapped (1). Chemicals aside from MLW were tested at identical molarities. Shading indicates bootstrapped 95% confidence intervals.

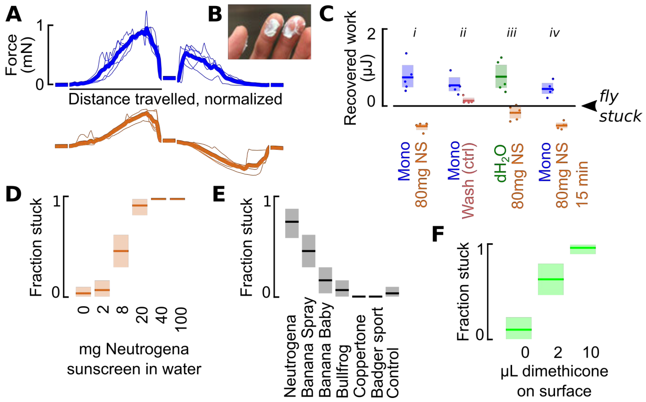


Figure 6. Oils, notably dimethicone (a common ingredient in sunscreens and cosmetics), annihilates alkali flies' super hydrophobic properties. (A) Force traces of 5 flies dipped into MLW (blue), as in 2A, and MLW containing dissolved Neutrogena Ultra Sheer spf 50 sunscreen (brown). To prepare the solution, 160 mg of sunscreen (B) was rubbed into both hands and allowed to set for 15 minutes. We then poured 300 mL of MLW over one hand, and used the run-off solution. **(B)** 160 mg sunscreen. **(C)** Work done on flies to escape MLW or pure water, with or without Neutrogena Ultrasheer spf 50 sunscreen (NS) run-off. *(i)* Sunscreen set for 5 minutes before rinsing with MLW. *(ii)* Hands thoroughly washed with soap and rinsed with warm water before rinsing. *(iii)* Sunscreen set for 5 minutes before rinsing with pure water. *(iv)* Sunscreen set for 15 minutes before rinsing with MLW. **(D)** Fraction of flies stuck to surface when dunked into pure water with increasing concentrations of Neutrogena sunscreen. N=30 flies for each condition, mean and 95% confidence intervals shown. **(E)** Fraction of flies stuck when dunked into pure water with different types of sunscreen (20 mg each). Same procedure as D. **(F)** To test whether dimethicone is sufficient to trap flies on the water's surface, we applied 0, 2, or 10 μL to the surface, and performed the same test described in D.

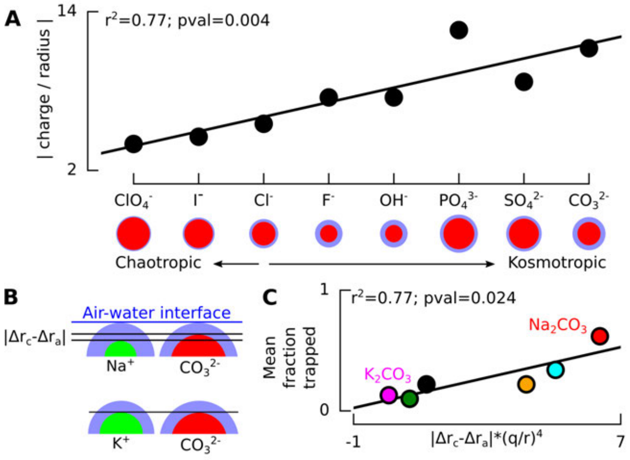


Figure 7. Ion specific interactions including relative hydration shell size and charge density help to explain ion facilitated wetting. (A) Hofmeister series of anions is correlated with the ratio of ion charge to radius. Black line shows the regression ($p=0.004$, $r^2=0.77$). Diagrams depict ions (red) and their hydration shells (blue), drawn to scale using the values reported in (24). **(B)** Comparison of Na^+ and K^+ ions to CO_3^{2-}. **(C)** Correlation of the fraction of flies stuck in solutions from Fig. 5 (mean across concentrations) with the product of the relative size of the cation and anion hydration shells and the ratio of the charge (q) and radius (r) of the ion closest to the air-water interface. Black line shows the regression ($p=0.024$, $r^2=0.77$).