

Table S1

This table contains data on the country of origin, collection date, global distribution, and laboratory of origin of the three *Drosophila* species used in this study.

Species	Origin	Collection date	Distribution	Source
<i>D. birchii</i>	Australia	2008	Tropical	Hoffmann, AUS
<i>D. melanogaster</i>	Denmark	2011	Cosmopolitan	Loescheke, DNK
<i>D. montana</i>	Finland	2008	Temperate	Hoikkala, FIN

Table S2

This table contains all results of statistical analyses comparing controls with treatment measurements of hindgut reabsorption of fluid and ions in all pharmacological interventions. Shown in the table is the treatment, what epithelia it was applied to, how the effect was tested, the compound the test was run on (fluid, K⁺ or Na⁺ reabsorption), the t-value, the degrees of freedom (df), and the resulting P-value. P < 0.05 is shown in bold.

Treatment	Applied to	Test	Compound	t	df	P-value
1 mmol L ⁻¹ NaCN	Hindgut, Malpighian tubules, fore- and midgut	Paired t test	Fluid	9.8	4	0.001
			K ⁺	5.9		0.004
			Na ⁺	7.2		0.002
1 mmol L ⁻¹ NaCN	Hindgut	Paired t test	Fluid	7.4	2	0.018
			K ⁺	4.1		0.055
			Na ⁺	8.3		0.014
1 mmol L ⁻¹ NaCN	Malpighian tubules, fore- and midgut	Paired t test	Fluid	14.7	2	0.005
			K ⁺	4.4		0.048
			Na ⁺	8.4		0.014
1 mmol L ⁻¹ ouabain	Hindgut	Paired t test	Fluid	3.3	4	0.029
			K ⁺	1.3		0.265
			Na ⁺	2.6		0.058
10 μmol L ⁻¹ bafilomycin A _I (2% DMSO)	Hindgut	Paired t test	Fluid	6.0	4	0.004
			K ⁺	1.1		0.350
			Na ⁺	5.4		0.006
2% DMSO	Hindgut	Paired t test	Fluid	3.6	2	0.070
			K ⁺	-3.3		0.081
			Na ⁺	1.7		0.237
100 μmol L ⁻¹ DIDS	Hindgut	Paired t test	Fluid	8.7	3	0.003
			K ⁺	9.6		0.002
			Na ⁺	15.0		0.001
1 mmol L ⁻¹ 8-bromo-cAMP	Hindgut, Malpighian tubules, fore- and midgut	Paired t test	Fluid	-4.8	7	0.002
			K ⁺	-4.3		0.002
			Na ⁺	-4.7		0.002
65 mmol L ⁻¹ Na ⁺ in saline	Hindgut, Malpighian tubules, fore- and midgut	Unpaired t test	Fluid *	1.7	40	0.091
			K ⁺ *	0.0		0.984
			Na ⁺ *	4.0		< 0.001

*The control values for reabsorption in the experiments involving lowered [Na⁺] in the saline consist of 36 measurements performed in the other experiments under the same conditions (i.e. control measurements for all other experiments presented in this table and the control measurements from the time control).

Table S3

The results of all statistical analyses performed to investigate the effect of 1) time, 2) species and test temperature, and 3) acclimation temperature and test temperature on reabsorption of fluid, K⁺, and Na⁺ are shown in table S3. Depicted is the dataset on which the analyses was performed, the dependent variable, the test used, the random/repeated factor, the dependent variable, the F- or t-value, the degrees of freedom (df), and the corresponding P-value. P < 0.05 is shown in bold.

Dataset	Variable	Test	Random effect	Factor(s)	F	t	df	P-value
Time series	Fluid reabsorption rate (nL min ⁻¹)	LMEM	Fly	Time*		-3.9	1,11	0.003
	K ⁺ reabsorption rate (pmol min ⁻¹)	LMEM	Fly	Time*		-2.7	1,11	0.019
	Na ⁺ reabsorption rate (pmol min ⁻¹)	LMEM	Fly	Time*		-5.1	1,11	< 0.001
Interspecific variation	Fluid reabsorption rate (nL min ⁻¹)	LMEM	Fly	Species	0.3	2,18	0.716	
				Temperature	122.0	1,18	< 0.001	
				Species x Temperature	1.4	2,18	0.279	
	K ⁺ reabsorption rate (pmol min ⁻¹)	LMEM	Fly	Species	25.2	2,18	< 0.001	
				Temperature	155.8	1,18	< 0.001	
				Species x Temperature	21.3	2,18	< 0.001	
Acclimation	Na ⁺ reabsorption rate (pmol min ⁻¹)	LMEM	Fly	Species	10.4	2,18	0.001	
				Temperature	143.8	1,18	< 0.001	
				Species x Temperature	0.6	2,18	0.552	
	Q ₁₀ (Fluid reabsorption)	ANOVA	-	Species	7.6	2,18	0.004	
	Q ₁₀ (K ⁺ reabsorption)	ANOVA	-	Species	1.3	2,18	0.308	
	Q ₁₀ (Na ⁺ reabsorption)	t test	-	Species (<i>D. montana</i> and <i>D. melanogaster</i>)	-1.8	12	0.096	
	Temperature effect ratio	ANOVA	-	Species	12.9	2,18	< 0.001	
	Fluid reabsorption rate (nL min ⁻¹)	LMEM	Fly	Acclimation temperature	3.5	2,22	0.050	
				Temperature	303.0	1,22	< 0.001	
				Acclimation temperature x Temperature	12.9	2,22	< 0.001	
	K ⁺ reabsorption rate (pmol min ⁻¹)	LMEM	Fly	Acclimation temperature	8.6	2,22	0.002	
				Temperature	190.1	1,22	< 0.001	
	Na ⁺ reabsorption rate (pmol min ⁻¹)	LMEM	Fly	Acclimation temperature x Temperature	7.9	2,22	0.003	
				Acclimation temperature	0.0	2,22	0.995	
				Temperature	208.4	1,22	< 0.001	
				Acclimation temperature x Temperature	6.1	2,22	0.008	
	Q ₁₀ (Fluid reabsorption)	ANOVA	-	Acclimation temperature	10.3	2,22	0.001	
	Q ₁₀ (K ⁺ reabsorption)	ANOVA	-	Acclimation temperature	13.0	2,22	< 0.001	
	Q ₁₀ (Na ⁺ reabsorption)	ANOVA	-	Acclimation temperature	18.5	2,22	< 0.001	
	Temperature effect ratio	ANOVA	-	Acclimation temperature	7.4	2,22	0.004	

*For the time series, time was included as a continuous variable instead of a factor.