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Supplemental information

Identification of a neural basis for cold acclimation

in *Drosophila* larvae

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Supplementary material from “Identification of a neural basis for cold acclimation in *Drosophila* larvae”

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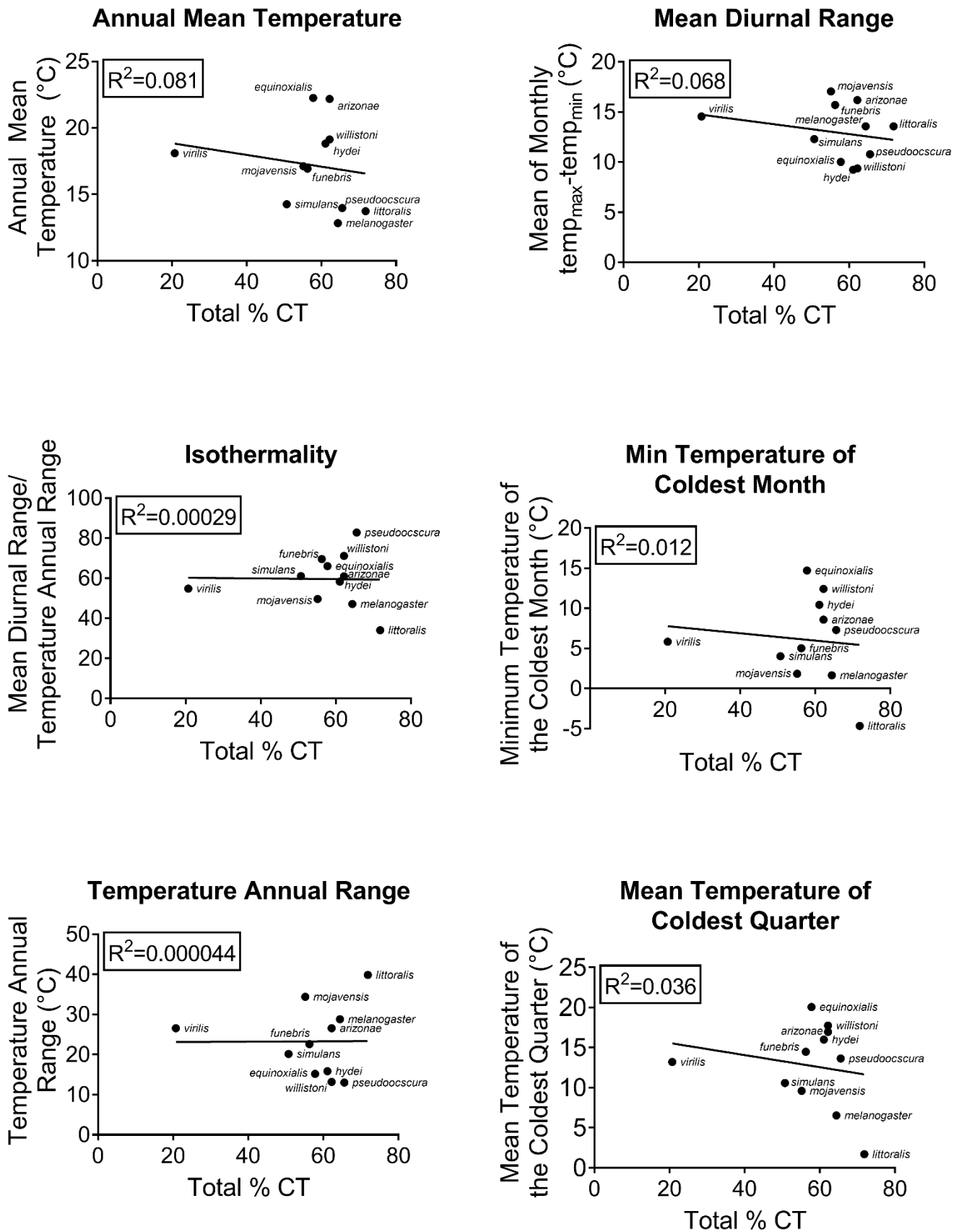


Figure S1. Correlations between environmental variables and Total % CT across temperatures, related to Figure 3.

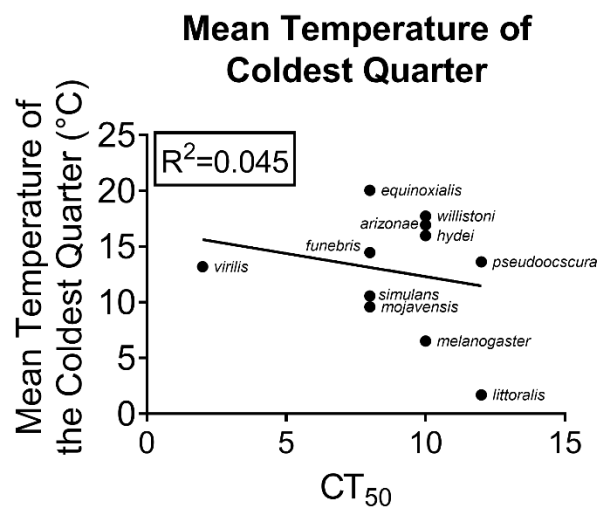
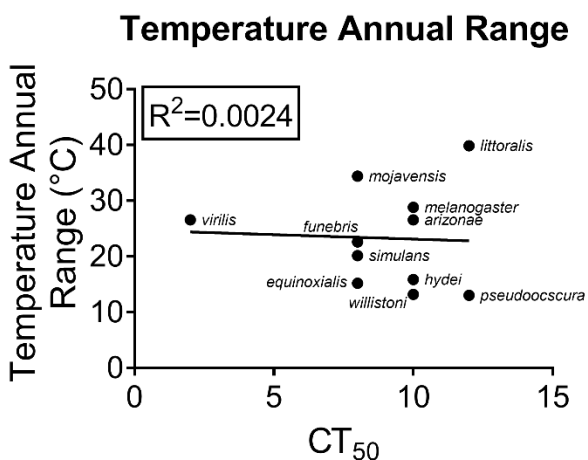
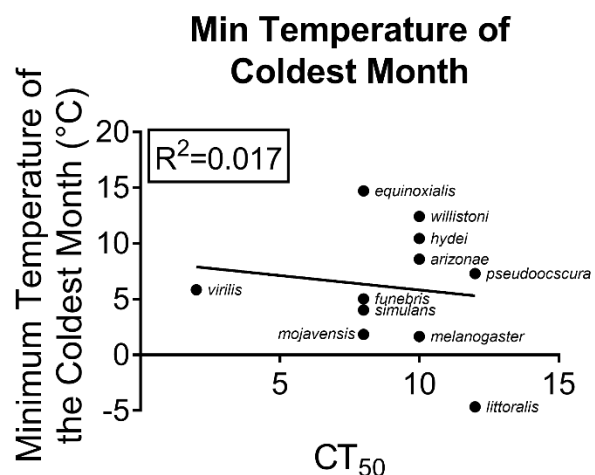
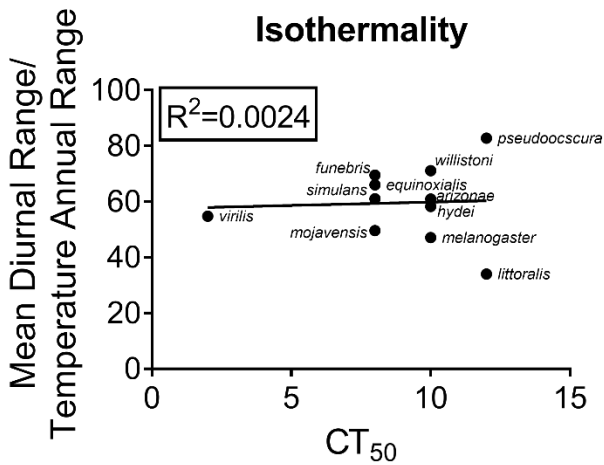
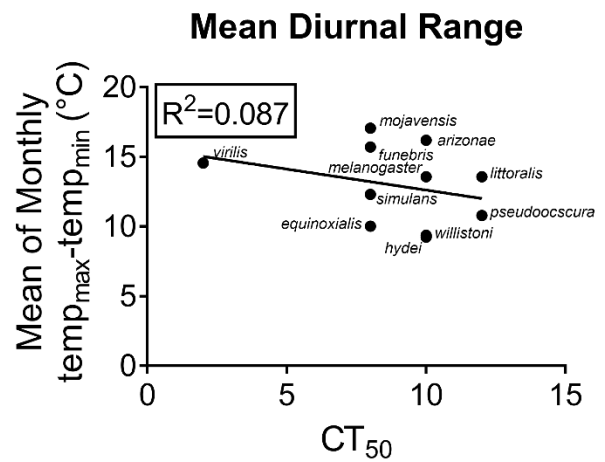
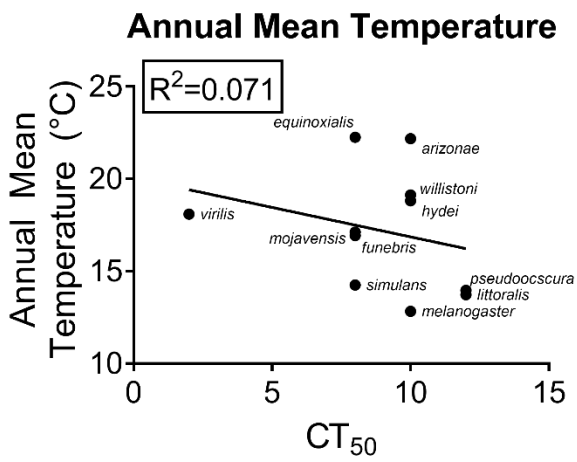


Figure S2. Correlations between environmental variables and temperature of $\geq 50\%$ subject CT (CT₅₀), related to Figure 3.

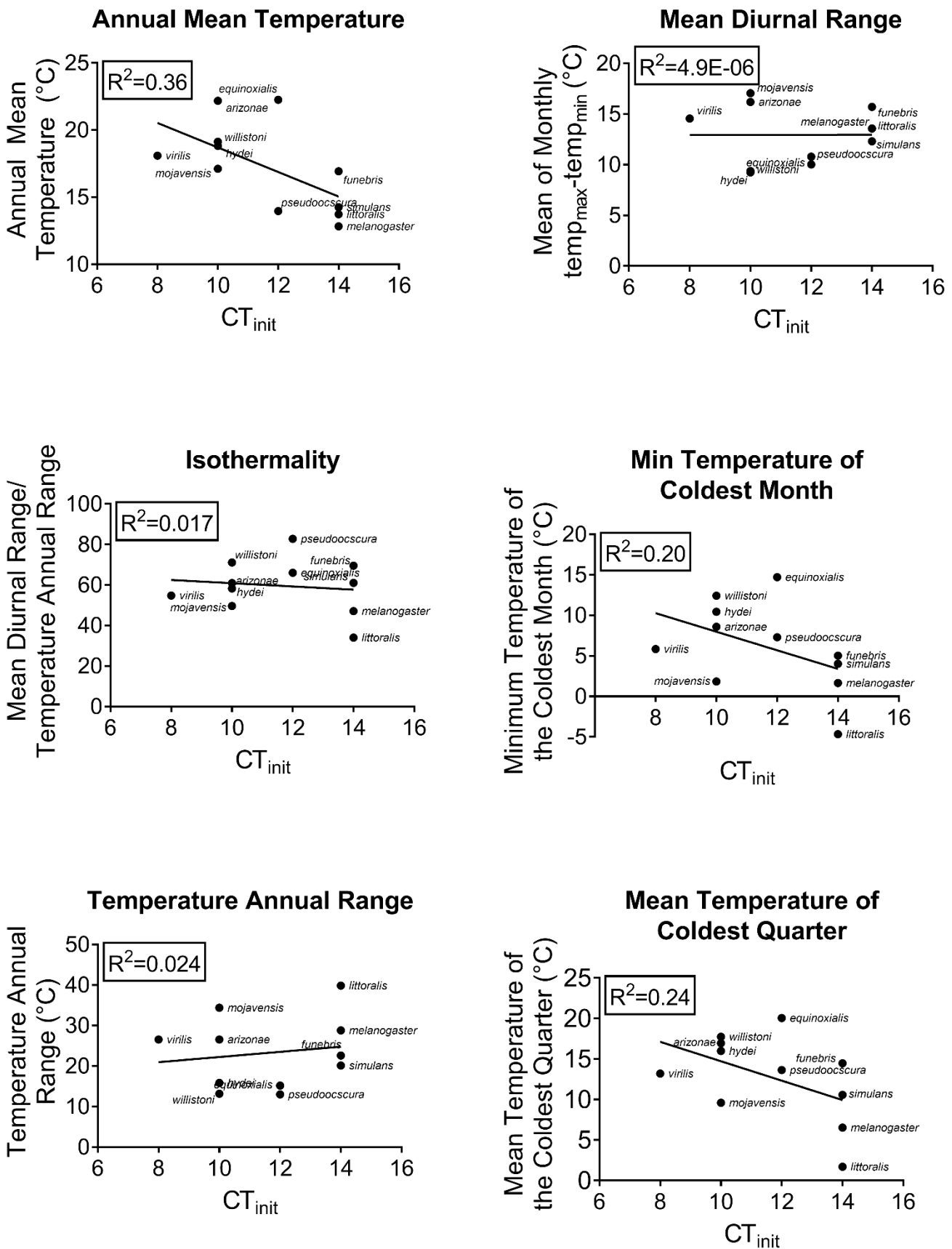


Figure S3. Correlations between environmental variables and highest temperature of CT onset, related to Figure 3.

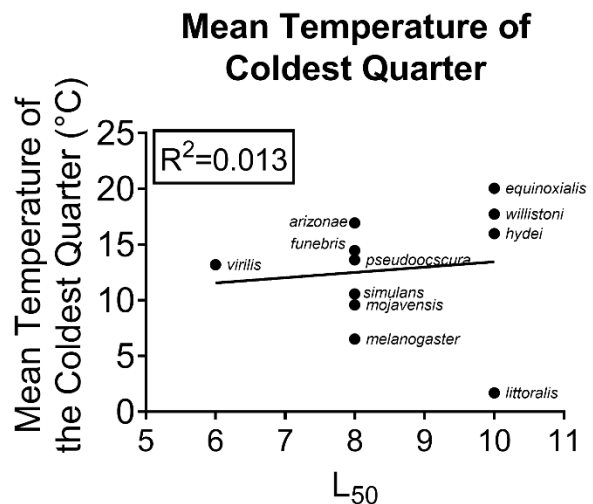
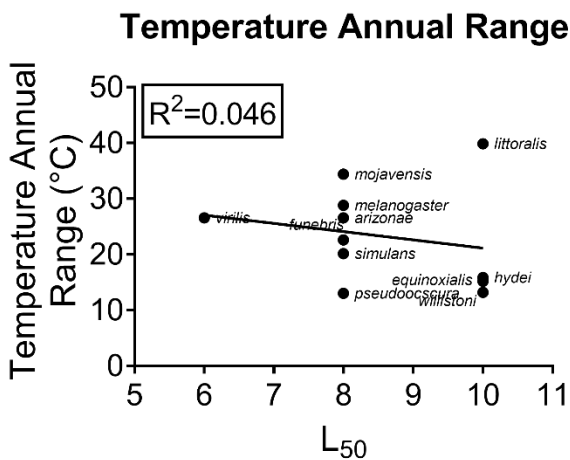
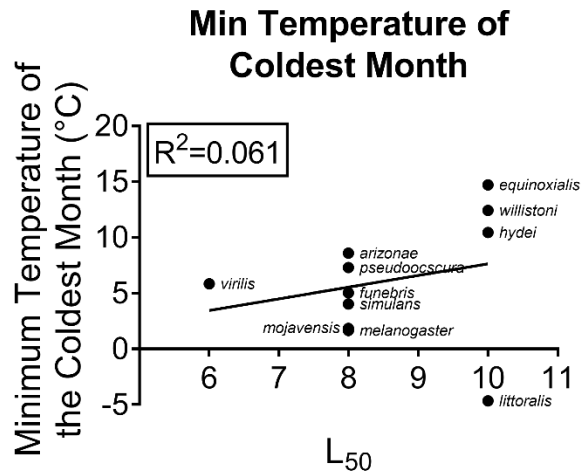
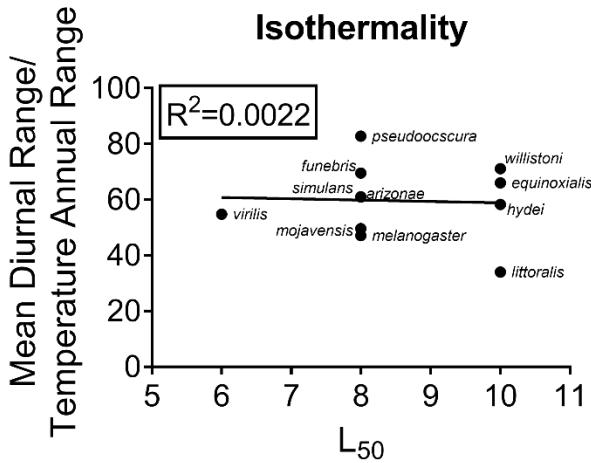
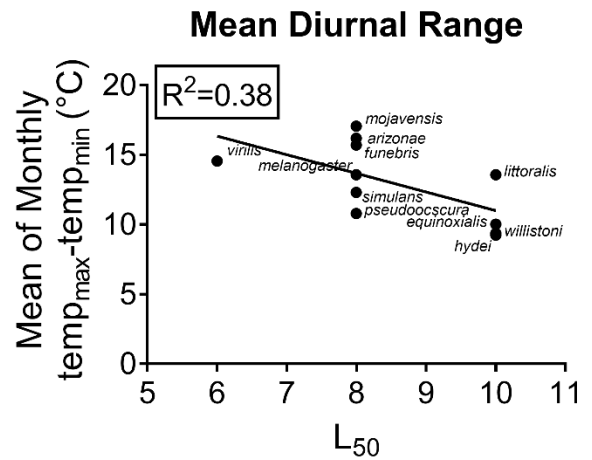
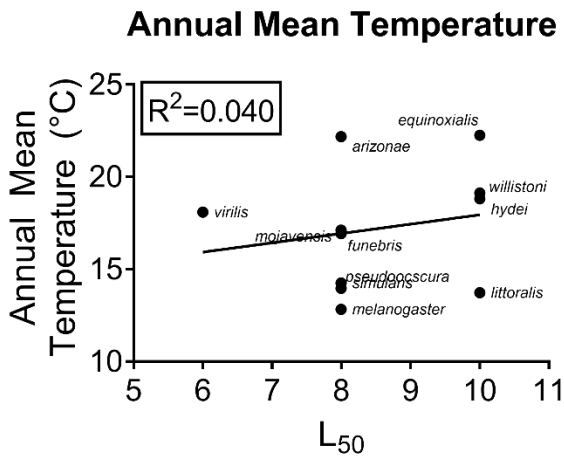


Figure S4. Correlations between environmental variables and temperature of $\leq 50\%$ subject locomotion (L_{50}), related to Figure 3

Binned CIII Cold-Evoked Activity

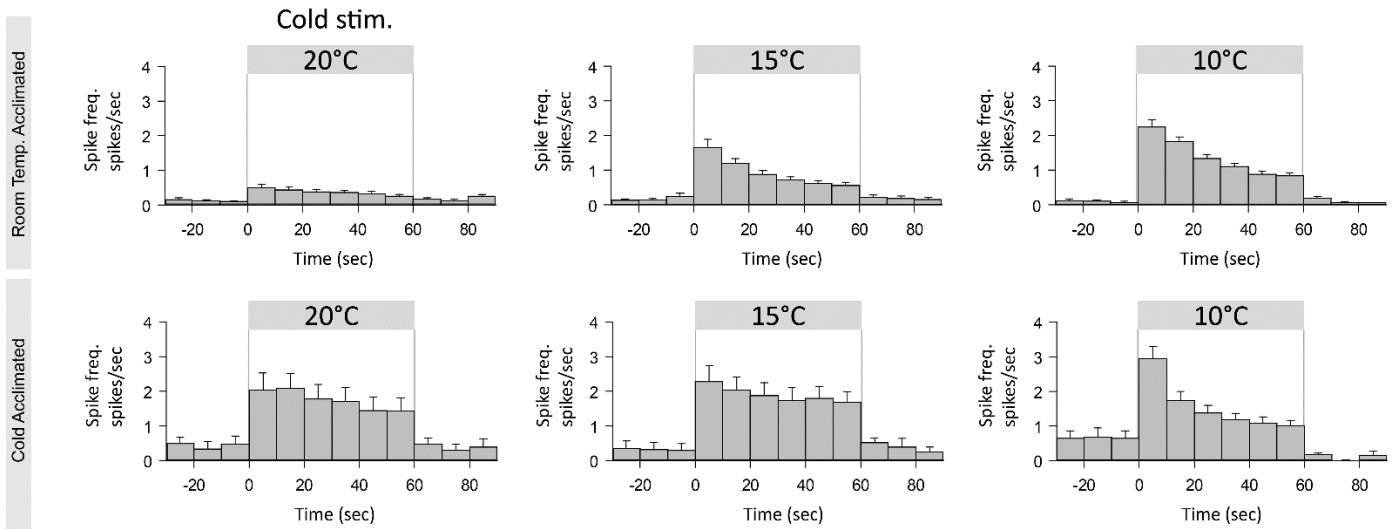


Figure S5. CIII neural activity by 10 second bins, related to Figure 5.

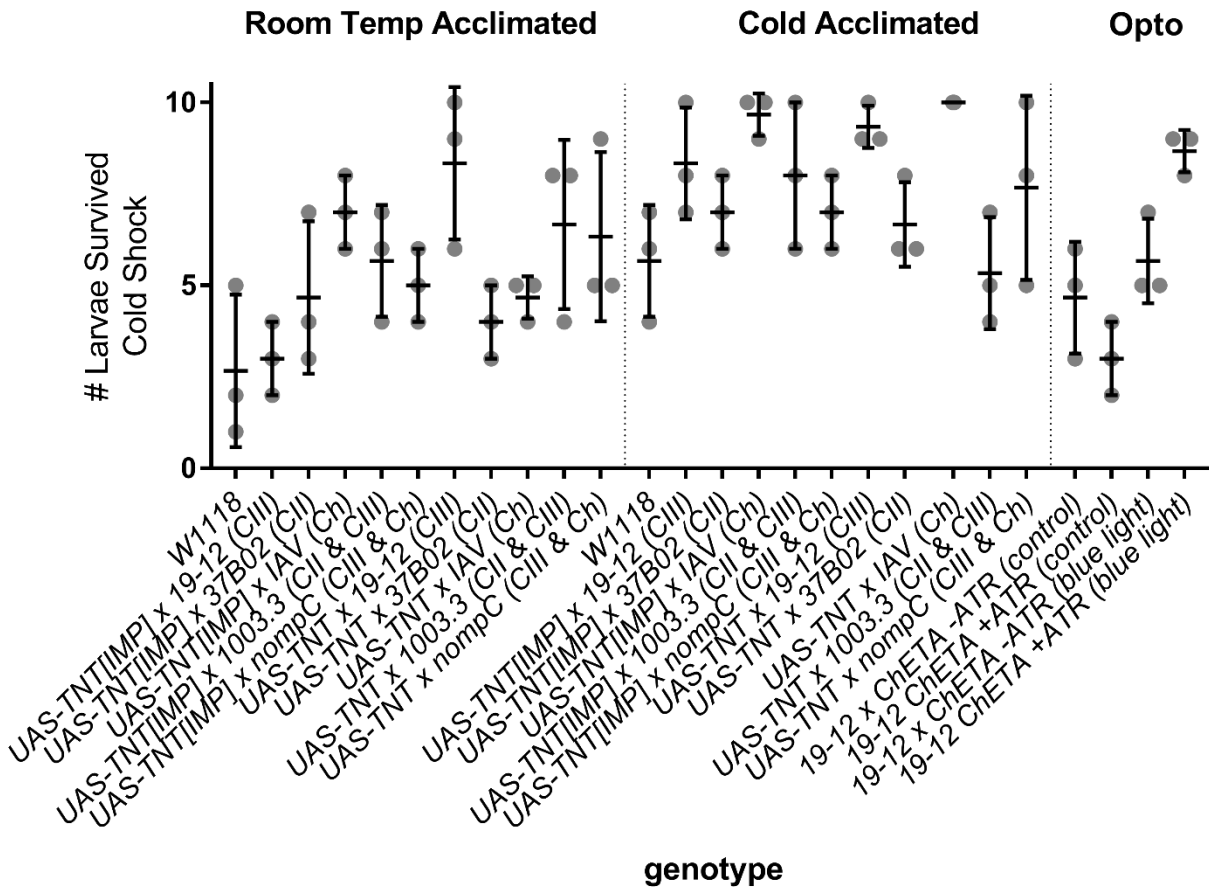


Figure S6. Plot of individual replicates for cold-shock survival experiments, related to Figure 4.

Species	Accession Number		
	Col	Col	Adh
<i>Drosophila melanogaster</i>	FBpp0100176	FBpp0100177	FBpp0100047
<i>Drosophila simulans</i>	NP_982323	P50253	XP_016024999
<i>Drosophila pseudoobscura</i>	YP_009742607	KAF3759836	XP_001357375
<i>Drosophila willistoni</i>	DAA06222	AAR05888	XP_015033661
<i>Drosophila equinoxialis</i>	AUW36081	AAL83280	Q9NG42
<i>Drosophila virilis</i>	ADW41404	ABD84161	XP_002057583
<i>Drosophila littoralis</i>	YP_002327403	YP_002327404	ABG56059
<i>Drosophila hydei</i>	AFV25509	AAD37708	P23236
<i>Drosophila funebris</i>	AHL43561	AAM70094	CAA73700
<i>Drosophila mojavensis</i>	DAA06235	ABD93167	XP_002002930
<i>Drosophila arizonae</i>	ABD38168	ABD93155	XP_017859111

Table S1. Accession numbers for sequences used to generate *Drosophila* phylogeny, related to Figure 1. Sequences from *Drosophila melanogaster* from FlyBase; all others from GenBank.

melanogaster

Temp	Loco	P	CT	SER	HR	TR	HTR	HW	twitch	roll
0	0%	0%	100%	0%	0%	0%	0%	0%	0%	0%
2	0%	0%	100%	0%	0%	0%	0%	0%	0%	0%
4	0%	0%	100%	0%	0%	0%	0%	0%	0%	0%
6	3%	0%	100%	0%	0%	0%	0%	0%	0%	0%
8	10%	0%	87%	0%	0%	0%	0%	3%	0%	0%
10	87%	7%	77%	0%	0%	7%	7%	13%	0%	0%
12	100%	83%	17%	0%	0%	0%	0%	0%	0%	0%
14	87%	0%	0%	0%	0%	0%	0%	0%	0%	0%
16	100%	0%	0%	0%	0%	0%	0%	0%	0%	0%

Table S2. *Drosophila melanogaster* cold-evoked behavioral program, related to Figure 1. Each value shows the % of larvae performing a particular behavior, at a particular temperature.

simulans

Temp	Loco	P	CT	SER	HR	TR	HTR	HW	twitch	roll
0	0%	3%	97%	0%	0%	0%	0%	0%	0%	0%
2	0%	0%	90%	0%	0%	0%	0%	10%	0%	0%
4	0%	7%	90%	0%	0%	0%	0%	3%	0%	0%
6	0%	0%	90%	0%	3%	17%	10%	0%	0%	0%
8	17%	0%	70%	0%	0%	13%	10%	0%	3%	0%
10	59%	45%	14%	0%	3%	3%	3%	14%	3%	0%
12	63%	87%	3%	0%	0%	7%	3%	3%	0%	0%
14	90%	83%	3%	0%	0%	0%	0%	0%	0%	0%
16	100%	0%	0%	0%	0%	0%	0%	0%	0%	0%

Table S3. *Drosophila simulans* cold-evoked behavioral program, related to Figure 1. Each value shows the % of larvae performing a particular behavior, at a particular temperature.

virilis

Temp	Loco	P	CT	SER	HR	TR	HTR	HW	twitch	roll
0	0%	0%	57%	0%	3%	0%	0%	0%	0%	7%
2	0%	0%	63%	0%	0%	0%	0%	0%	0%	0%
4	0%	0%	47%	0%	0%	0%	0%	0%	0%	0%
6	0%	0%	17%	0%	0%	3%	3%	0%	0%	0%
8	7%	7%	3%	0%	7%	0%	0%	0%	0%	0%
10	70%	67%	0%	0%	0%	3%	3%	0%	0%	0%
12	73%	67%	0%	0%	0%	0%	0%	0%	0%	0%
14	100%	20%	0%	0%	0%	0%	0%	0%	0%	0%
16	100%	0%	0%	0%	0%	0%	0%	0%	0%	0%

Table S4. *Drosophila virilis* cold-evoked behavioral program, related to Figure 1. Each value shows the % of larvae performing a particular behavior, at a particular temperature.

pseudoobscura

Temp	Loco	P	CT	SER	HR	TR	HTR	HW	twitch	roll
0	0%	0%	100%	0%	7%	0%	0%	0%	0%	0%
2	0%	0%	100%	0%	10%	0%	0%	0%	0%	0%
4	0%	0%	100%	0%	20%	0%	0%	0%	0%	0%
6	0%	0%	97%	0%	0%	0%	0%	0%	0%	0%
8	50%	0%	97%	0%	3%	0%	0%	0%	0%	0%
10	77%	7%	90%	0%	0%	0%	0%	0%	0%	0%
12	93%	77%	7%	0%	0%	0%	0%	0%	0%	0%
14	87%	27%	0%	0%	0%	0%	0%	0%	0%	0%
16	100%	0%	0%	0%	0%	0%	0%	0%	0%	0%

Table S5. *Drosophila pseudoobscura* cold-evoked behavioral program, related to Figure 1. Each value shows the % of larvae performing a particular behavior, at a particular temperature.

willistoni

Temp	Loco	P	CT	SER	HR	TR	HTR	HW	twitch	roll
0	0%	0%	97%	0%	0%	3%	0%	0%	0%	0%
2	0%	0%	90%	0%	0%	0%	0%	0%	0%	3%
4	0%	0%	90%	0%	0%	7%	0%	0%	0%	0%
6	0%	0%	93%	0%	0%	3%	0%	0%	0%	0%
8	0%	0%	100%	0%	0%	0%	0%	0%	0%	0%
10	0%	0%	90%	0%	3%	0%	0%	0%	0%	0%
12	87%	87%	0%	0%	0%	0%	0%	0%	0%	0%
14	100%	100%	0%	0%	0%	0%	0%	0%	0%	0%
16	100%	50%	0%	0%	0%	0%	0%	0%	0%	0%

Table S6. *Drosophila willistoni* cold-evoked behavioral program, related to Figure 1. Each value shows the % of larvae performing a particular behavior, at a particular temperature.

equinoxialis

Temp	Loco	P	CT	SER	HR	TR	HTR	HW	twitch	roll
0	0%	0%	100%	0%	0%	10%	3%	0%	0%	0%
2	0%	0%	100%	0%	0%	17%	13%	0%	0%	0%
4	0%	0%	100%	0%	0%	0%	0%	0%	0%	0%
6	0%	0%	73%	0%	7%	3%	0%	7%	3%	3%
8	0%	0%	80%	0%	10%	3%	3%	10%	3%	3%
10	10%	14%	48%	0%	0%	7%	3%	31%	0%	0%
12	87%	40%	20%	0%	0%	3%	3%	27%	0%	7%
14	100%	67%	0%	0%	0%	0%	0%	0%	7%	0%
16	100%	80%	0%	0%	0%	0%	0%	0%	0%	0%

Table S7. *Drosophila equinoxialis* cold-evoked behavioral program, related to Figure 1. Each value shows the % of larvae performing a particular behavior, at a particular temperature.

funnebris

Temp	Loco	P	CT	SER	HR	TR	HTR	HW	twitch	roll
0	0%	0%	100%	0%	0%	0%	0%	0%	0%	0%
2	0%	0%	100%	0%	7%	0%	0%	0%	0%	0%
4	0%	0%	83%	0%	3%	0%	0%	10%	0%	0%
6	13%	0%	97%	0%	0%	0%	0%	3%	0%	0%
8	13%	0%	97%	0%	0%	0%	0%	0%	0%	0%
10	90%	14%	17%	0%	0%	0%	0%	24%	0%	0%
12	100%	0%	10%	0%	3%	0%	0%	20%	0%	0%
14	100%	3%	3%	0%	0%	0%	0%	0%	0%	0%
16	100%	0%	0%	0%	0%	0%	0%	0%	0%	0%

Table S8. *Drosophila funnebris* cold-evoked behavioral program, related to Figure 1. Each value shows the % of larvae performing a particular behavior, at a particular temperature.

arizonae

Temp	Loco	P	CT	SER	HR	TR	HTR	HW	twitch	roll
0	0%	0%	100%	0%	0%	10%	3%	0%	0%	0%
2	0%	0%	100%	10%	0%	3%	3%	0%	0%	0%
4	0%	0%	100%	23%	0%	20%	13%	0%	0%	0%
6	0%	0%	100%	87%	10%	30%	0%	0%	3%	0%
8	27%	0%	93%	10%	3%	3%	0%	3%	0%	7%
10	53%	30%	67%	0%	0%	0%	0%	3%	0%	0%
12	60%	90%	0%	0%	0%	0%	0%	10%	0%	0%
14	93%	33%	0%	0%	0%	0%	0%	17%	0%	0%
16	100%	3%	0%	0%	0%	0%	0%	0%	0%	0%

Table S9. *Drosophila arizonae* cold-evoked behavioral program, related to Figure 1. Each value shows the % of larvae performing a particular behavior, at a particular temperature.

littoralis

Temp	Loco	P	CT	SER	HR	TR	HTR	HW	twitch	roll
0	0%	3%	100%	0%	10%	0%	0%	0%	0%	0%
2	0%	17%	100%	0%	10%	3%	0%	0%	0%	0%
4	0%	0%	100%	0%	10%	0%	0%	0%	0%	0%
6	13%	0%	100%	0%	3%	10%	0%	0%	0%	0%
8	20%	3%	93%	0%	0%	0%	0%	0%	0%	0%
10	37%	7%	90%	0%	3%	3%	0%	0%	0%	0%
12	63%	30%	50%	0%	0%	0%	0%	0%	0%	0%
14	87%	57%	13%	0%	0%	3%	0%	0%	0%	0%
16	100%	20%	0%	0%	3%	0%	0%	0%	0%	0%

Table S10. *Drosophila littoralis* cold-evoked behavioral program, related to Figure 1. Each value shows the % of larvae performing a particular behavior, at a particular temperature.

mojavensis

Temp	Loco	P	CT	SER	HR	TR	HTR	HW	twitch	roll
0	0%	0%	100%	30%	3%	27%	10%	0%	0%	0%
2	0%	0%	93%	17%	0%	23%	7%	0%	0%	0%
4	0%	0%	97%	3%	0%	20%	0%	0%	0%	0%
6	3%	3%	97%	40%	3%	67%	0%	0%	0%	0%
8	37%	3%	80%	13%	0%	0%	0%	10%	0%	0%
10	70%	13%	30%	3%	10%	0%	0%	40%	0%	3%
12	93%	0%	0%	0%	3%	0%	0%	0%	0%	0%
14	100%	0%	0%	0%	3%	0%	0%	0%	0%	3%
16	100%	0%	0%	0%	0%	0%	0%	0%	0%	0%

Table S11. *Drosophila mojavensis* cold-evoked behavioral program, related to Figure 1. Each value shows the % of larvae performing a particular behavior, at a particular temperature.

hydei

Temp	Loco	P	CT	SER	HR	TR	HTR	HW	twitch	roll
0	0%	0%	100%	70%	0%	0%	0%	0%	0%	0%
2	0%	0%	100%	33%	0%	0%	0%	0%	0%	0%
4	0%	0%	100%	43%	0%	0%	0%	0%	0%	0%
6	0%	0%	100%	30%	0%	17%	0%	0%	0%	0%
8	3%	0%	97%	0%	0%	0%	0%	3%	0%	0%
10	37%	7%	53%	30%	0%	0%	0%	20%	0%	0%
12	77%	87%	0%	3%	0%	0%	0%	10%	0%	0%
14	93%	10%	0%	0%	0%	0%	0%	0%	0%	0%
16	100%	0%	0%	0%	0%	0%	0%	0%	0%	0%

Table S12. *Drosophila hydei* cold-evoked behavioral program, related to Figure 1. Each value shows the % of larvae performing a particular behavior, at a particular temperature.