

Figure S1

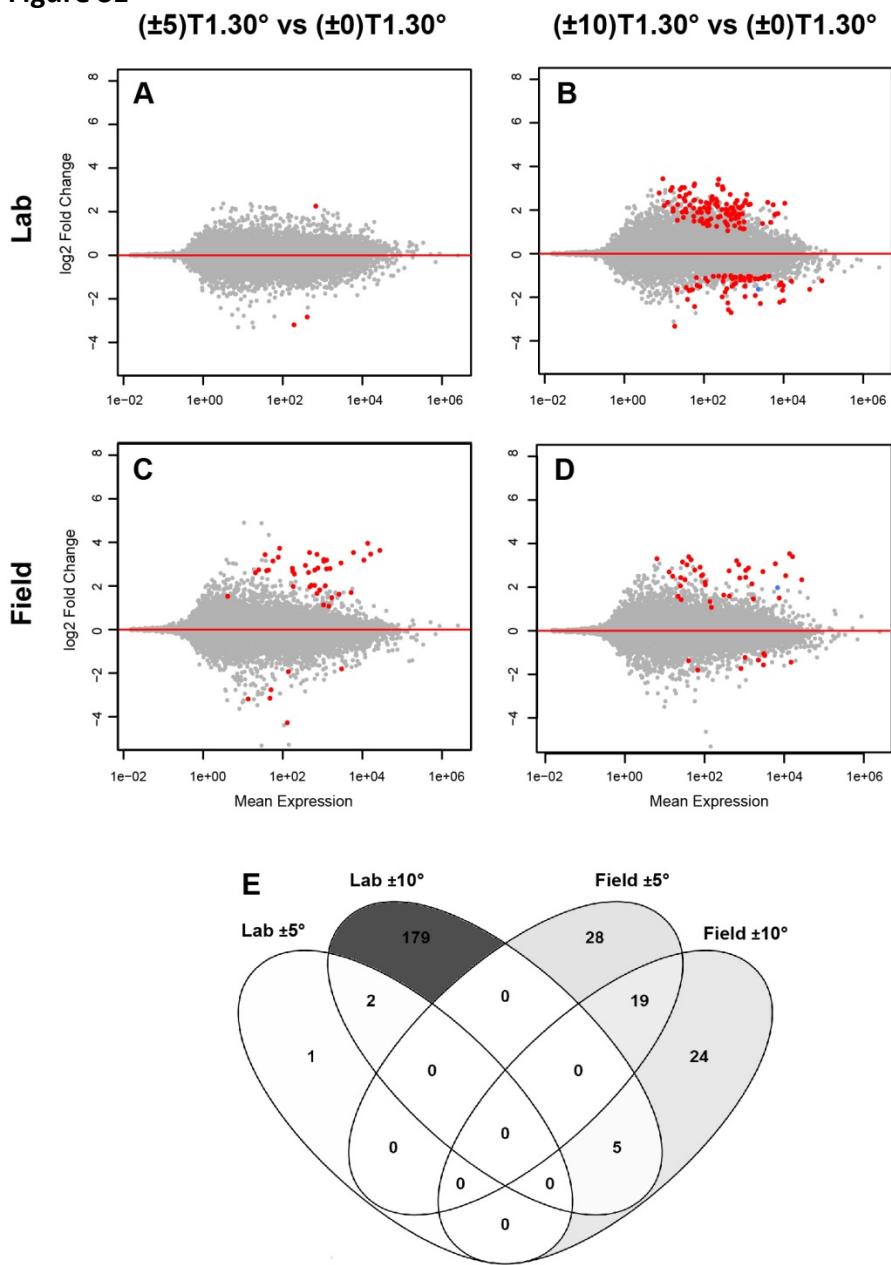


Figure S1. Differential Gene Expression - Fluctuating vs Constant Rearing Comparisons

(A-D) MA plots showing differentially expressed (DE) genes for lab (A, B) and field (C, D) *M. sexta* populations after development in fluctuating rearing conditions of 30 ± 5 (A, C) or $30\pm 10^\circ\text{C}$ (B, D) compared to a constant ($30\pm 0^\circ\text{C}$) control. All treatments were sampled at 30°C , at time point 1 (see figure 1). Significant genes (FDR < 0.01 & LFC > 1) are highlighted in red and significant *hsp* genes are shown in blue. **(E)** Venn diagram of shared significantly DE genes from these four treatment comparisons (fluctuating vs constant rearing). Shading indicates relative proportion of genes in each set.

Table S1. Numbers of Significantly DE Genes between Populations

Field vs lab pairwise comparisons	Total	↑/↓	# HSPs ↑/↓
(±0)T1.30°	610	398/212	1/2
(±5)T1.30°	1185	852/333	1/2
(±10)T1.30°	213	139/74	0/2
(±0)T2.30°	329	199/130	0/1
(±0)T2.35°	1262	765/497	7/1
(±0)T2.40°	857	422/435	4/2
(±5)T2.35°	2475	1400/1075	3/5
(±10)T2.40°	369	206/163	0/1

Table S1. Numbers of significantly DE genes between populations for each of the pairwise treatment comparisons separately, including: total, number of total that are up- and down-regulated respectively, and number of total that are annotated as *hsp* genes (up- and down-regulated *hsp* genes also shown separately). Direction of change in expression (up/down) calculated for first treatment listed relative to second treatment. Significance was assessed using a FDR cutoff of < 0.01 and a LFC in expression > 1.

Table S2. Gene ontology (GO) enrichment results organized by pairwise treatment comparison. Only pairwise comparisons that had enrichment results are included.

GO category names: BP (biological process), CC (cellular component), MF (molecular function)
All enrichment results for a given pairwise treatment comparison are included for FDR > 0.1 however formally significant results are indicated by bolding and asterisk(s) to denote the level of significance: * indicates FDR < 0.05 and ** indicates FDR < 0.01.

Methods for Field vs Lab comparison: List of significant DE genes for field vs lab population comparison was calculated using a different model in DESeq2 (negative binomial model accounting for only for population and not temperature treatment effects).

Table S2. GO Enrichment Results

GO Cat.	GO Name	FDR	# Genes	Expression Change	GO ID
Field vs Lab Population (across temperature treatments)					
BP	DNA integration	2.11E-17**	38	up	GO:0015074
BP	DNA metabolic process	2.44E-09**	55	up	GO:0006259
BP	chitin metabolic process	3.90E-02*	12	up	GO:0006030
MF	iron ion binding	1.72E-03**	25	up	GO:0005506
MF	oxidoreductase activity, acting on paired donors	5.09E-03**	24	up	GO:0016705
MF	monooxygenase activity	7.47E-03**	17	up	GO:0004497
MF	heme binding	2.68E-02*	23	up	GO:0020037
--	genes without GO terms	1.70E-79**	418	down	--
Rearing: (± 10)T1.30° vs (± 0)T1.30°, Lab Population					
BP	folic acid-containing compound biosynthesis	3.88E-04**	4	up	GO:0009396
BP	proteolysis	1.34E-02*	15	up	GO:0006508
BP	10-formyltetrahydrofolate metabolic process	1.62E-02*	2	up	GO:0009256
BP	negative regulation of endopeptidase activity	9.86E-02	3	up	GO:0010951
BP	cellular modified amino acid catabolic process	9.92E-02	2	up	GO:0042219
CC	extracellular matrix	4.99E-05**	6	up	GO:0031012
CC	collagen trimer	7.46E-04**	3	up	GO:0005581
MF	formate-tetrahydrofolate ligase activity	1.62E-02*	2	up	GO:0004329
MF	extracellular matrix structural constituent	9.11E-02	2	up	GO:0005201
MF	GTP cyclohydrolase I activity	6.93E-02	2	up	GO:0003934
MF	hydroxymethyl-, formyl- and related transferase activity	9.11E-02	2	up	GO:0016742
MF	L-ascorbic acid binding	9.11E-02	2	up	GO:0031418
MF	chitin binding	8.44E-02	4	up	GO:0008061
MF	peptidase activity, acting on L-amino acid peptides	9.86E-02	12	up	GO:0070011
Novel Heat Shock: (± 0)T2.35° vs (± 0)T2.30°, Lab Population					
BP	signal peptide processing	1.50E-05**	4	up	GO:0006465
BP	mRNA splicing, via spliceosome	1.50E-05**	8	up	GO:0000398
BP	nuclear-transcribed mRNA catabolic process	2.93E-02*	3	up	GO:0000956
BP	protein sumoylation	5.48E-02	2	up	GO:0016925
BP	protein targeting	7.86E-02	4	up	GO:0006605
BP	protein N-linked glycosylation	8.85E-02	2	up	GO:0006487
CC	ribonucleoprotein complex	4.57E-04**	14	up	GO:0030529
CC	signal peptidase complex	4.76E-04**	3	up	GO:0005787
CC	U4/U6 x U5 tri-snRNP complex	1.07E-02*	3	up	GO:0046540
CC	U6 snRNP	2.19E-02*	2	up	GO:0005688
CC	mitochondrion	3.39E-02*	8	up	GO:0005739

CC	spliceosomal complex	6.86E-02	3	up	GO:0005681
BP	lipid transport	6.04E-06**	9	down	GO:0006869
BP	Wnt signaling pathway	4.07E-05**	7	down	GO:0016055
BP	proteolysis	4.07E-05**	26	down	GO:0006508
BP	tetrahydrofolate biosynthetic process	3.34E-03**	3	down	GO:0006508
BP	metabolic process	3.34E-03**	36	down	GO:0044710
BP	semaphorin-plexin signaling pathway	6.05E-03**	3	down	GO:0071526
BP	biosynthetic process	7.30E-03**	14	down	GO:0044711
BP	folic acid biosynthetic process	1.45E-02*	3	down	GO:0046656
BP	10-formyltetrahydrofolate metabolic process	1.89E-02*	2	down	GO:0009256
BP	carbohydrate metabolic process	3.57E-02*	11	down	GO:0005975
BP	transport	4.12E-02*	11	down	GO:0044765
BP	'de novo' IMP biosynthetic process	4.67E-02*	2	down	GO:0006189
BP	localization	4.75E-02*	11	down	GO:1902578
BP	cell adhesion	5.67E-02	5	down	GO:0007155
BP	glycosphingolipid metabolic process	5.78E-02	3	down	GO:0006687
BP	oxidation-reduction process	6.94E-02	18	down	GO:0055114
CC	extracellular region	4.07E-05**	19	down	GO:0005576
MF	lipid transporter activity	1.19E-08**	8	down	GO:0005319
MF	catalytic activity	3.63E-07**	86	down	GO:0003824
MF	serine-type endopeptidase activity	3.19E-04**	15	down	GO:0004252
MF	lipid binding	7.52E-04**	8	down	GO:0008289
MF	GTP cyclohydrolase I activity	1.58E-03**	3	down	GO:0003934
MF	hydrolase activity, hydrolyzing O-glycosyl compounds	3.28E-03**	9	down	GO:0003934
MF	hydroxymethyl-, formyl- and related transferase activity	3.34E-03**	3	down	GO:0016742
MF	semaphorin receptor activity	6.05E-03**	3	down	GO:0017154
MF	formate-tetrahydrofolate ligase activity	1.89E-02*	2	down	GO:0004329
MF	transmembrane transporter activity	2.62E-02*	13	down	GO:0022892
MF	calcium ion binding	4.73E-02	9	down	GO:0005509
MF	oxidoreductase activity	8.64E-02	18	down	GO:0016491

Novel Heat Shock: (± 0)T2.35° vs (± 0)T2.30°, Field Population

--	genes without GO terms	5.42E-02	143	up	--
BP	chitin metabolic process	1.27E-02*	8	up	GO:0006030
MF	chitin binding	8.89E-02	7	up	GO:0008061
MF	catalytic activity	1.30E-03**	49	down	GO:0003824
MF	monooxygenase activity	6.78E-02	6	down	GO:0004497

Novel Heat Shock: (± 0)T2.40° vs (± 0)T2.30°, Lab Population

BP	proton transmembrane transport	4.94E-05**	13	up	GO:0006818
BP	purine ribonucleoside metabolic process	1.64E-03**	13	up	GO:0046128

BP	transport	3.73E-03**	18	up	GO:0044765
BP	localization	4.71E-03**	18	up	GO:1902578
BP	cellular biosynthetic process	2.97E-02*	46	up	GO:0044249
BP	mitochondrial electron transport, cytochrome c to oxygen	4.10E-02*	4	up	GO:0006123
BP	protein maturation	7.00E-02	4	up	GO:0051604
BP	pyrimidine-containing compound metabolic process	7.81E-02	6	up	GO:0072527
CC	nucleoplasm part	2.56E-02*	11	up	GO:0044451
CC	respiratory chain complex III	3.20E-02*	3	up	GO:0045275
CC	respiratory chain complex IV	4.10E-02*	4	up	GO:0045277
CC	mitochondrial proton-transporting ATP synthase complex	6.06E-02	3	up	GO:0005753
CC	endoplasmic reticulum part	9.15E-02	6	up	GO:0044432
MF	ubiquinol-cytochrome-c reductase activity	2.13E-02*	3	up	GO:0008121
MF	NADH dehydrogenase activity	2.31E-02*	4	up	GO:0003954
MF	ATPase activator activity	3.70E-02*	2	up	GO:0001671
MF	SUMO transferase activity	3.70E-02*	2	up	GO:0019789
MF	cytochrome-c oxidase activity	4.10E-02*	4	up	GO:0004129
MF	DNA-directed 5'-3' RNA polymerase activity	4.57E-02*	5	up	GO:0003899
MF	palmitoyl-(protein) hydrolase activity	8.73E-02	2	up	GO:0008474
BP	biosynthetic process	4.04E-06**	27	down	GO:0044711
BP	DNA replication initiation	3.33E-03**	4	down	GO:0006270
BP	isoleucine biosynthetic process	3.60E-03**	3	down	GO:0009097
BP	valine biosynthetic process	3.60E-03**	3	down	GO:0009099
BP	leucine biosynthetic process	3.60E-03**	3	down	GO:0009098
BP	aminophospholipid transport	3.460E-03**	3	down	GO:0015917
BP	metabolic process	6.03E-03**	52	down	GO:0044710
BP	cellular localization	1.15E-02*	12	down	GO:0051641
BP	purine nucleobase metabolic process	1.48E-02*	9	down	GO:0006144
BP	tetrahydrofolate metabolic process	3.77E-02*	3	down	GO:0046653
BP	ncRNA processing	4.87E-02*	7	down	GO:0034470
BP	folic acid-containing compound biosynthetic process	5.07E-02	3	down	GO:0009396
BP	inositol phosphate biosynthetic process	5.50E-02	2	down	GO:0032958
BP	phenylalanyl-tRNA aminoacylation	5.50E-02	2	down	GO:0006432
BP	'de novo' IMP biosynthetic process	5.50E-02	2	down	GO:0006189
BP	dicarboxylic acid metabolic process	5.82E-02	5	down	GO:0043648
BP	protein localization	7.16E-02	10	down	GO:0008104
BP	vesicle-mediated transport	8.84E-02	8	down	GO:0016192
BP	gene silencing by RNA	8.84E-02	2	down	GO:0031047
CC	MCM complex	9.22E-04**	4	down	GO:0042555
CC	replication fork	3.30E-03**	6	down	GO:0005657
CC	clathrin coat of trans-Golgi network vesicle	2.28E-02*	2	down	GO:0030130

CC	retromer, cargo-selective complex	2.28E-02*	2	down	GO:0030906
CC	phenylalanine-tRNA ligase complex	5.50E-02	2	down	GO:0009328
CC	mitochondrial inner membrane peptidase complex	5.50E-02	2	down	GO:0042720
CC	DNA polymerase complex	7.50E-02	3	down	GO:0042575
CC	transcription factor complex	9.23E-02	11	down	GO:0005667
MF	catalytic activity	4.04E-06**	129	down	GO:0003824
MF	aminoacyl-tRNA ligase activity	3.60E-03**	7	down	GO:0016876
MF	purine ribonucleoside binding	3.60E-03**	38	down	GO:0032550
MF	phospholipid-translocating ATPase activity	3.60E-03**	3	down	GO:0004012
MF	aminoacyl-tRNA editing activity	3.60E-03**	3	down	GO:0002161
MF	ATP binding	3.60E-03**	33	down	GO:0005524
MF	hydroxymethyl-, formyl- and related transferase activity	7.36E-03**	3	down	GO:0016742
MF	magnesium ion binding	1.20E-02*	5	down	GO:0000287
MF	carbon-nitrogen ligase activity, with glutamine as amido-N-donor	1.29E-02*	3	down	GO:0016884
MF	DNA-binding transcription factor activity	1.29E-02*	12	down	GO:0003700
MF	DNA binding	1.72E-02*	26	down	GO:0003677
MF	formate-tetrahydrofolate ligase activity	2.28E-02*	2	down	GO:0004329
MF	DNA-directed DNA polymerase activity	5.07E-02	3	down	GO:0004329
MF	organic anion transmembrane transporter activity	5.50E-02	2	down	GO:0008514
MF	phenylalanine-tRNA ligase activity	5.50E-02	2	down	GO:0004826
MF	DNA helicase activity	5.50E-02	4	down	GO:0003678
MF	chromatin binding	8.84E-02	3	down	GO:0003682

Novel Heat Shock: (± 0)T2.40° vs (± 0)T2.30°, Field Population

BP	DNA replication	2.58E-04**	8	down	GO:0006260
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Thermal History: (± 0)T2.35° vs (± 5)T2.35°, Field Population

--	genes without GO terms	9.88E-02	99	up	--
BP	chitin metabolic process	5.48E-05**	9	up	GO:0006030
MF	chitin binding	6.78E-04**	8	up	GO:0008061
MF	calcium ion binding	9.31E-02	10	up	GO:0005509
BP	defense response	2.50E-03**	5	down	GO:0006952
BP	defense response to fungus	5.10E-03**	3	down	GO:0050832
BP	cell surface pattern recognition receptor signaling pathway	3.80E-02*	2	down	GO:0002752
BP	response to stress	5.76E-02	5	down	GO:0006950
BP	response to external stimulus	6.61E-02	3	down	GO:0009605
CC	extracellular region	3.11E-02*	7	down	GO:0005576
MF	signaling pattern recognition receptor activity	3.80E-02*	2	down	GO:0008329
MF	carbohydrate binding	4.38E-02*	3	down	GO:0030246

Table S3. Novel Heat Shock Shared GO Enrichment Results

Shared Comparisons	GO Name	Expression Change
All	HSPs Various metabolic processes	Up Down
35 °C (Lab and Field)	Catalytic activity	Down
40 °C (Lab and Field)	DNA replication	Down
Lab Population (35 and 40 °C)	Mitochondrial components/respiration Sumoylation Amino /nucleic acid biosynthesis Carbohydrate metabolism Transport/transporter activity	Up UP Down Down Down
Field Population (35 and 40 °C)	None	--

Table S3. Enriched gene ontology (GO) terms that were shared by two or more novel heat shock comparisons ((±0)T2.35° vs (±0)T2.30° or (±0)T2.40° vs (±0)T2.30°, either population).

Table S4 Novel Heat Shock Comparisons Shared DE Genes

Gene ID	Top BLAST Hit(s)
Up	
Msex2.00685	dnaJ homolog subfamily A member 1 [<i>Spodoptera litura</i>]
Msex2.00968	hsp 21.5a [<i>Choristoneura fumiferana</i>]
Msex2.00970	hsp 19.9 [<i>Antheraea pernyi</i>]
Msex2.01805	hsp 23.7 [<i>Bombyx mori</i>]
Msex2.01818	hsp 20.4 [<i>Bombyx mori</i>]
Msex2.01819	hsp 20.8 [<i>Bombyx mori</i>]
Msex2.01822	hsp 19.7 [<i>Chilo suppressalis</i>]; hsp 19.9 [<i>Bombyx mori</i>]
Msex2.03056	hsp 12.2 [<i>Bombyx mori</i>]; 19.5 kDa hsp [<i>Bombyx mori</i>]
Msex2.04632	uncharacterized protein [<i>Helicoverpa armigera</i>]; vacuolar protein-sorting machinery protein hse1-like isoform X2 [<i>Bombyx mandarina</i>]
Msex2.05066	protein windbeutel [<i>Bombyx mori</i>]
Msex2.07879	uncharacterized protein [<i>Helicoverpa armigera</i>]
Msex2.10127	hsp 70 [<i>Manduca sexta</i>]; hsp 70 A1 [<i>Bombyx mori</i>]
Msex2.10129	hsp 22.1 [<i>Choristoneura fumiferana</i>]
Msex2.10327	farnesoate epoxidase precursor [<i>Bombyx mori</i>]
Msex2.10432	protein SGT1 homolog [<i>Galleria mellonella</i>]
Msex2.11262	thioredoxin-like protein 4A [<i>Bombyx mori</i>]
Msex2.12807	hsp 68 isoform X1 [<i>Bombyx mori</i>]
Msex2.13903	hsp 70 [<i>Manduca sexta</i>]; hsp 70 A1 [<i>Bombyx mori</i>]
Msex2.15235	F-box only protein 28 [<i>Galleria mellonella</i>]
Down	
Msex2.03228	transient receptor potential channel pyrexia-like [<i>Spodoptera litura</i>]
Msex2.05116	inositol polyphosphate multikinase [<i>Bombyx mori</i>]
Msex2.08138	choline/ethanolamine kinase [<i>Ostrinia furnacalis</i>]
Msex2.08825	ATP-binding cassette sub-family B member 6, mitochondrial [<i>Helicoverpa armigera</i>]

Table S4. List of 23 significantly differentially expressed (DE) genes shared among all four novel heat shock comparisons ((±0)T2.35° vs (±0)T2.30° and (±0)T2.40° vs (±0)T2.30°, both populations).