



s o c o e i n s S a n g e f o m o a e a e s n e s i e i i o s l i n e a o i c a n o a o i c c e l l s i n e s o n s e o e a a n o e s s e s s e s i n c e e m o l e a n c e i n s o m e o g a n i s m s | A s o n g c o e l a i o n a s e e n f o n e e e n S e e s s i o n a n e m o l e a n c e | e e e s s i o n a n i n o c i o n o f e o g e n o s S i n c e a s e e e m o l e a n c e o f a i o s e s o f m a m m a l i a n c e l l s i n c l e o e c s c e l l s a g a i n s l a i o l e a i a i o n o e c s o l e m a m m a l i a n e a s a g a i n s o s i s c e m i c a m a a n i n c e a s e e i n c i l e e m o l e a n c e o f *Drosophila* c e l l s i n c l e e m o s a n l a a e | n c e e m o l e a n c e i s m e i a e i n c e a s e e e s s i o n o f e a s o c o e i n s i n a i e a i e o f c e l l s a n o g a n i s m s | a n g a n a n g | s i e e g e n e i c a s i s o f e m o l e a n c e i n o i c a l a n e m e a e o l a i o n s o f e m i g a o l o c s *Locusta migratoria* m e a s i n g e e s s i o n o f S a n S m A a l o ° C a n i g e m e a e s ° C a n s g g e s e a e m o l e a n c e o f l o c s e g g s a a c o m l e g e n e i c a s i s a n e a s o c o e i n s m i g e i n o l e i n i f f e e n c e s i n e m o l e a n c e e e e n l o c s o l a i o n s s o l e m e n i o n e a e c o n i o n i n g i n s e c s c a n c o n f e e m a l o l e a n c e o a s s e e n i g e e m a l e a m e n s c a n e f f e c t i s n o n e c e s s a r i l e l a e o S s o e f a c o s m a a i c i a e i n e m a l o l e a n c e |

a n i l a i o n o e n g i n e e i n g o f g e n e s e l a e o e m o l e a n c e a s e o e f o e c o n m e o f S i c a s s f f i c i e n t o a f f e c t i n c i l e e m o l e a n c e a s o m e l i f e s a g e s o f *Drosophila melanogaster* | e *Drosophila* e a s o c o e i n S o m o e a s i n o c e a s i e f o i n c i l e e e s s i o n o f e o g e n o s g e n e s i n i n s e c s a n s c c e s s f l a n s f e c i o n i e n e *Drosophila* S o m o e a s c a i e o f o e l o i n g a e a s o c i n c i l e a n a n i n e i a l e A i n e f e n c e A i s s e m i n e s i l o m *B. mori* |

o m a n n a n i i f o i n i c a e a e e a s o c e s o n s e o f *B. mori* a s s i m i l a o a o f o e i n s e c s i n i c o c e e e e g o s o f e a s o c o e i n s i n c l i n g e S S a n s o f a c c o i n g o m o l e c l a e i g m a e s o n e i m e n s i o n a l g e l e l e c o o e s i s e a l s o c o n c l e a e e a s o c e s o n s e o f *B. mori* a s i f f e e n a n a o f *Drosophila* i n i c e e e s s i o n o f n o n e a s o c o e i n s n e s i s i n g e a s o c a s n o a o m i n e n f e a e o f e e s o n s e s i n g e e a s o c e s o n s e o f e i f f e e n a c e s o f s i l o m i n c l i n g e m l i o l i n e e e s C i c i a n e s o e a n e i o l i n e e e o a n o i n a a n s o e a e e s e n c e o f n e o e i n s i n e s o n s e o e a s o c a s i f f e e n a m o n g i f f e e n i s s e s a n a o m l i o l i n e a n i o l i n e s i l o m s e s o n e o e a s o c a s e i e n c e e e s e n c e o f a i o n a l o e i n s

a s e o e a e e s s i o n o f e a s o c o e i n s i n s i l o m m i g a i n i f f e e n e l o m e n a l s a g e s a s e o n e e i m e n s i n g S S A e l e c o o e s i s | i e t a l . | a n a l e e e e s s i o n o f e s m a l l e a s o c g e n e m S i n s i l o m s C a n f o n a i n g l e e l s o f i s o e i n i n i s s e s a s m o s a n a n i n e s i s o a s i l g l a n a n a e S o n g e t a l . | f o n a e

e a s o c a o e i n c o g n a e a s o n e o f e e g l a e e m o c i c o e i n s e n s i l o m l a a e s o n e o e i n o c l a i o n o f e a i n a c i a e a c e i a *Bacillus megaterium*

S c a l o e o m i c s m a s o e s c e o f o e i n c o m l e e s o e o e i n s e s e n i n g i n a s e c i f i c c e l l l a o g a n e l l e f n c i o n a l o e o m i c s i s a o a e m f o m a n s e c i f i c i e c e o e o m i c s a o a c e s a n e e s s i o n o e o m i c s i s e a n i a i e s o f o e i n e e s s i o n i n s a m l e s a i f f e i n s o m e a i a l e | e e a e e e n n o l i c a i o n s o a e e o i n g e o e o m i c s a o a c o e a s o c o e i n s i n s i l o m n e e s e n o o o e o m e a e n s o f f a o f o m e a s o c e s i l o m s a s c o m a e i c o n o l s i l o m s i n o e s i s a n a n s s c e i l e e e s a n e e s s i o n a e n s o f e i f f e e n i a l o e i n e e a g e e f o i e n i f i c a i o n e f a o i s s e o f i n s e c s a o m o l o g e o f m a m m a l i a n l i e a s i m o a n f n c i o n s a s a s o a g e i s s e a n a s a e c e n e o f m e a o l i s m a n i o c e m i s

## MATERIALS AND METHODS

### e e i c a e r i a s a d k p r m e a r i

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### erma rea me s a d k a m i

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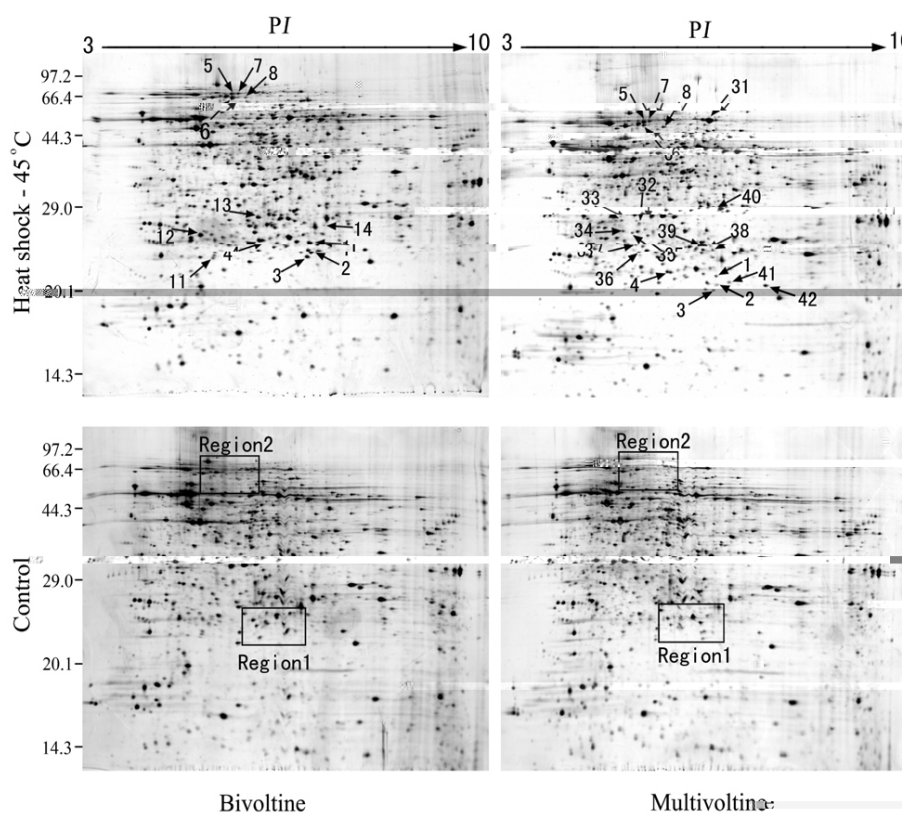
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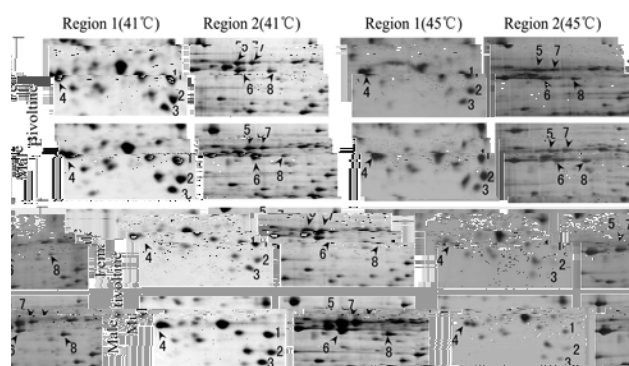
## RESULTS

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**Fig. 1.** 2D electrophoresis protein profiles of fat body of the control and heat exposed silkworm larvae from the thermo-susceptible a 24L0gAnhkNg0goP0OggoNOz



**Table 1.** List of identified silkworm fat body proteins in responses to high heat exposure

Spot no.	Protein name (Matched organism)	Accession GI no.	No. of peptides (coverage)	Protein score (C.I. %)	Mr calcd/obsd (PI calcd/obsd)	Ontology
<b>Common response spots</b>						
1	Heat shock protein HSP20.4 ( <i>Bombyx mori</i> )	49036077	9 (49.86%)	148 (100)	26 / 20.41 (7.10 / 6.54)	Response to stress
2	DNA-formamidopyrimidine glycosylase* (alpha proteobacterium BAL199)	163793016	7 (31.00%)	90 (99.42)	25 / 32.87 (7.05 / 8.57)	Zinc ion binding, DNA binding; catalytic activity
3	Heat shock protein HSP 19.9 ( <i>Bombyx mori</i> )	56378317	7 (29.24%)	120 (100)	24 / 19.88 (6.23 / 6.53)	Response to stress
4	Heat shock protein HSP20.8 ( <i>Bombyx mori</i> )	11120618	7 (46.09%)	177 (100)	26 / 20.79 (5.80 / 5.98)	Response to stress
5	Heat shock protein HSP70 ( <i>Antheraea yamamai</i> )	47232576	16 (40.28%)	98 (99.96)	85 / 69.55 (5.80 / 5.7)	Response to stress
6	Heat shock protein HSP70 ( <i>Antheraea yamamai</i> )	47232576	12 (25.87%)	110 (99.98)	80 / 69.55 (5.9 / 5.7)	ATP binding
7	Heat shock protein HSP70 ( <i>Antheraea yamamai</i> )	47232576	13 (29.65%)	102 (99.98)	85 / 69.55 (5.9 / 5.7)	Response to stress
8	Heat shock protein HSP70 ( <i>Antheraea yamamai</i> )	47232576	15 (45.40%)	86 (99.44)	79 / 69.55 (6.15 / 5.7)	ATP binding
<b>Specific response spots (Bivoltine)</b>						
11	Heat shock protein HSP20.1* ( <i>Bombyx mori</i> )	112983134	7 (33.00%)	84 (97.80)	25 / 20.18 (5.51 / 5.46)	Response to stress
13	PREDICTED: similar to zinc finger protein 436 ( <i>Canis familiaris</i> )	57048379	7 (20.54%)	80 (97.74)	30 / 55.30 (6.3 / 8.94)	Zinc ion binding
<b>Specific response spots (Multivoltine)</b>						
34	PREDICTED: similar to CG10504-PA* ( <i>Tribolium castaneum</i> )	91079909	14 (33.00%)	82 (96.20)	33 / 51.45 (5.45 / 7.77)	Transferase activity; protein amino acid phosphorylation
36	Heat shock protein HSP21.4 ( <i>Bombyx mori</i> )	56378321	8 (60.43%)	120 (100)	29/2139 (5.74 / 5.79)	Response to stress
38	PREDICTED: similar to zinc finger protein 46* ( <i>Canis familiaris</i> )	57048379	13 (27.00%)	96 (99.84)	29/55.30 (6.91 / 8.94)	Zinc ion binding
40	PREDICTED: similar to CG9935-PA isoform 1* ( <i>Apis mellifera</i> )	66507549	11 (27.00 %)	86 (98.50)	37/61.90 (7.04 / 6.14)	Transferase activity; protein amino acid phosphorylation

C.I. %: confidence interval of protein score.

\*Identification of protein by PMF analysis.

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**Table 2.** The mean of normalized volumes (%) of 8 protein spots, including 4 sHSP (region 1) and 4 HSP70 (region 2), in different treatments, breeds, and sexes

Breed	Sex	Heat treatment (45°C)			Heat treatment (41°C)	
		Number of spot*	sHSP	HSP70	sHSP	HSP70
Bivoltine	Female	534	0.353 (± 0.102)	0.215 (± 0.086)	0.322 (± 0.067)	0.218 (± 0.063)
	Male	744	0.332 (± 0.091)	0.225 (± 0.070)	0.332 (± 0.069)	0.221 (± 0.135)
Multivoltine	Female	582	0.072 (± 0.043)	0.151 (± 0.050)	0.282 (± 0.063)	0.278 (± 0.221)
	Male	825	0.077 (± 0.040)	0.225 (± 0.079)	0.235 (± 0.042)	0.302 (± 0.040)

\*Total number of spots in 2D electrophoresis image pattern.

**Table 3.** ANOVA on normalized volumes of 8 protein spots including 4 sHSP (region 1) and 4 HSP70 (region 2)

Source	df	sHSP		HSP70	
		M.S.	P	M.S.	P
Heat treatment	1	0.057	0.008	0.021	0.125
Breed	1	0.226	0.000	0.003	0.554
Sex	1	0.001	0.657	0.006	0.400
Error	28	0.007	–	0.008	–

in the intensity of protein expression between the safe 45°C and the 41°C treatments. No significant differences were observed in the expression of the 8 protein spots between the two breeds and between the two sexes. The ANOVA results (Table 3) showed that the heat treatment had a significant effect on the expression of the 8 protein spots (p = 0.008). The breed and sex had no significant effect on the expression of the 8 protein spots (p = 0.554 and p = 0.400, respectively). The error term was not significant (p = 0.125).

## DISCUSSION

The mal sensibility of the silkworms to the heat shock is a well-known fact. The silkworms are very sensitive to the heat shock, and the heat shock can cause the death of the silkworms. The heat shock can also cause the silkworms to produce a large amount of heat shock proteins (HSPs). The HSPs are a family of proteins that are produced in response to various stressors, including heat shock. The HSPs are involved in many cellular processes, including protein folding, protein degradation, and cell signaling. The HSPs are also involved in the response to heat shock. The HSPs are produced in response to heat shock, and they help the silkworms to survive the heat shock. The HSPs are also involved in the response to other stressors, such as oxidative stress and heavy metal stress. The HSPs are a family of proteins that are produced in response to various stressors, and they play a crucial role in the survival of the silkworms.

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The silkworms are very sensitive to the heat shock, and the heat shock can cause the death of the silkworms. The heat shock can also cause the silkworms to produce a large amount of heat shock proteins (HSPs). The HSPs are a family of proteins that are produced in response to various stressors, including heat shock. The HSPs are involved in many cellular processes, including protein folding, protein degradation, and cell signaling. The HSPs are also involved in the response to heat shock. The HSPs are produced in response to heat shock, and they help the silkworms to survive the heat shock. The HSPs are also involved in the response to other stressors, such as oxidative stress and heavy metal stress. The HSPs are a family of proteins that are produced in response to various stressors, and they play a crucial role in the survival of the silkworms.



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e e ession of s S s in e m l i o l i n e e e is sig nifican l < lo e an in e i o l i n e e e en e ose o e °C ea men e e is no iffe ence e een e e s en e ose o e °C ea men a le an i g is emons a es a e e mo ole an sil o m e e as no c a a c e i e a i g e l e el of s S s n esis n e s e e e a s oc as com a e o e e mosensi i e e e S e ession as no significan l e ce a e i g e e m e a e ea men is s gges s e s S an S ma la iffe en ole in e mo ole ance of sil o m s a s e on e a aila le esea c e a o s concl e a o e mec anisms mig e in ol e i e mo ole ance o e an e s S s an e S e n m e of s e c i f i c o e i n s in ol e in e ole ance of m l i o l i n e e e mig also a e an im o an ole as e a e e c e s o s in e m l i o l i n e e e com a e i s o s in e i o l i n e e e en e ose o ea S ilo a *et al.* | concl e a e mo ole ance e i e s e e a l a n a i e molec la mec anisms an a S s S s an o e ni en ifie fac o s la e an im o an ole in is ocess along i S in *D. melanogaster* e i o s esea c as s o n a in e mo ole an e e s of *D. melanogaster* S s n esis is main aine a lo le els e mos e mo ole an s ain isola e in Cen al Afica as a lo e le el of S s n esis n e mo e a e e a e os e °C com a e o e less e mo ole an e gon s ain |

i g an a le s o a male sil o m la a e esse slig l mo e S es eciall in e m l i o l i n e e e e iffe ence is no significan < e n m e of o e i n s o s e e c e image anal sis sof a e is also i g e in males an in females o e es of o l no le ge no o e lica ion isc sses e iffe ences e een female an male sil o m s in es on ing o ea ole ance e e e imen a ion is e i e o e e mine e iffe ence in e mo ole ance e een e s e s of sil o m la a e

n o e o i e n i f mo e o e i n m a e s an o e n an ce o n e s an ing of e ela ions i e een sil o m e e s an e i iffe en e mal ole ances an e i e essions of iffe en l in s of S s i is necessa o sea c fo mo e iffe en ial s o s sing mo e e mo ole an an s sce i le sil o m e e s A i o n a l m e o s fo e o e sil o m iss ess o l also e e lo e n e f e e e f o e e ill in es i g a e e effec s of e cessi e ea s o c on e o e o m e of iffe en e e s an s e s

e mo e e e a e man s ccessf l e e imen s on ansgenic sil o m o e e i is onl ecen l a scien is s a e ec nicall ca a le of a ge ing en o geno s genes en enginee ing ansgenic sil o m | e e f o e m a ni la ion of genes ela e o o s ness an e mo ole ance of sil o m is no oo fa a a An n e s an ing of e molec la mec anisms of e mal ole ance is essen ial fo a aining an es l s in is i ec ion a i c l a l in e n e s an ing e iffe en ial e ession a e n of a i o s S s in i o l i n e an m l i o l i n e e e s e im o an ce of S i c as confi me fo sil o m la a e

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## Acknowledgements

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