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Proteome Anal sis on Differentiall E pressed Proteins of the Fat Bod of T o Silk orm Breeds, *Bomb mori*, E posed to Heat Shock E posure

S. Hossein Hosseini Moghaddam[1,2], Xin Du[1], Jun Li[1], Jinru Cao[3], Boxiong Zhong[1], and YuYin Chen[1]*

[1] College of Animal Sciences e iang Uni e is ang o C ina
[2] Ag ic l e ac l Uni e si of ilan as an
[3] Se ic l al esea c ns i e e iang Aca em of Ag ic l al Sciences ang o C ina

Abstract Proteomes of heat tolerant (multivoltine) and heat susceptible (bivoltine) silkworms (*Bombyx mori*) in response to heat shock were studied. Detected proteins from fat body were identified by using MALDI-TOF/TOF spectrometer, MS/MS, and MS analysis. Eight proteins, including small heat shock proteins (sHSPs) and HSP70, were expressed similarly in both breeds, while 4 protein spots were expressed specifically in the bivoltine breed and 12 protein spots were expressed specifically in the multivoltine breed. In the present proteomics approach, 5 separate spots of sHSP proteins (HSP19.9, HSP20.1, HSP20.4, HSP20.8, and HSP21.4) were identified. Protein spot intensity of sHSPs was lower in the multivoltine breed than in the bivoltine breed after the 45°C heat shock treatment, while the difference between two breeds was not significant after the 41°C heat shock treatment. These results indicated that some other mechanisms might be engaged in thermal tolerance of multivotine breed except for the expression of sHSP and HSP70. There were visible differences in the intensity of heat shock protein expression between male and female, however, differences were not statistically significant.

Keywords: proteome analysis, heat shock proteins, silkworm, 2D electrophoresis, mass spectrometry

INTRODUCTION

e se ic l e in s as con i e significan l o e economic e elo men of man co n ies. is as e s l e in en ic e sil o m ge lasm eso ces e o e effo s of sil o m ee e s in e esea c of gene ics an ee ing of sil o m *Bombyx mori*. *B. mori* is se i el in asic esea c in io ec nolog an as a mo el insec . n e s of geog a ical aces an gene icall im o e s ains a e main aine in iffe en co n ies e e se ic l e as/is in og e. ese aces an s ains a e no onl in ell c a ac e i e en elian ai s also in no so ell s ie an i a i e ai s s c as o si e fee ing a ion e mal ole ance an isease esis ance . e mo ole ance is a ic la l em asi e in sil o m ee ing og ams fo e selec ion of sil o m a ie ies i e e a a a ion o a ie en i onmen al con i ions es eciall in ical egions s c as So e n n ia o s mme an a mn season in C ina.

A se of o eins no n as ea s oc o eins S s a ea s o e in ol e in ole ance agains a e se g o con i ion in man o ganisms . S s a e in ce a ai o s em e a es in iffe en o ganisms an e n m e of o eins an e ela i e im o ance of eac S famil in s ess ole ance a f om o ganism o o ganism . e concen a ion of in ci le S s an o e molec la c a e ones i in cells a . as een ell es a lis e a io e os e o s ess in ces ole ance an c oss ole ance o s se en s ess . S e ession can e co ela e i esis ance o s ess . i in e S s small ea

***Corresponding author**
Tel: +86-571-86971657 Fax: +86-571-86971657
e-mail: chenyy@z e cn

s oc o eins s S ange f om o a. e a e s n esi e i i o sl in e a o ic an o a o ic cells in es onse o ea an o e s esses in ce e mo ole ance in some o ganisms 7 . A s ong co ela ion as een fo n e een S 7 e ession an e mo ole ance . O e e ession an in o c ion of e ogeno s S 7 inc eases e e mo ole ance of a io s es of mammal ian cells in c l e o ec s cells agains l a iole a ia ion o ec s ole mammalian ea s agains os isc emic a ma an inc eases e in ci le e mo ole ance of *Drosophila* cells in c l e em os an la ae 1 . n ce e mo ole ance is me ia e inc ease e ession of ea s oc o eins in a i e a ie of cells an o gan isms 7 . ang an ang s ie e gene ic asis of e mo ole ance in ical an em e a e la ions of e mig a o loc s *Locusta migratoria* meas ing e ession of S 7 an S m A a lo °C an ig em e a es °C an s gges e a e mo ole ance of loc s eggs a a com le gene ic asis an ea s oc o eins mig e in ol e in iffe ences in e mo ole ance e een loc s la ions. s o l e men ione a e con i ioning insec s can confe e mal ole ance o a s se en ig e mal ea men s c an effec is no necessa il ela e o S s o e fac o s ma a ic a e in e mal ole ance .

am la ion o enginee ing of genes ela e o e mo ole ance as o e fo e co n m e of S 7 ic as s fficien o affec in ci le e mo ole ance a some life s ages of *Drosophila melanogaster* . e *Drosophila* ea s oc o ein 7 S 7 omo e as in o ce as i e fo in ci le e ession of e ogeno s genes in insec s an s ccessf l ansfec ion i en e *Drosophila* S 7 omo e as ca ie o fo e el ing a ea s oc in ci le an an in e i a le A in e fe ence Ai s s em in e sil o m *B. mori* .

o mann an i ifo in ica e a e ea s oc es onse of *B. mori* as simila o a of o e insec s in ic o ce e e ee g o s of ea s oc o eins incl ing e S S 7 an s S acco ing o mo lec la eig ma e s one imensional gel elec o esis . e also concl e a e ea s oc es onse of *B. mori* as iffe en an a of *Drosophila* in ic e es sion of non ea s oc o ein s n esis ing ea s oc as no a ominen fea e of e es onse. s ing e ea s oc es onse of e iffe en aces of sil o m in cl ing e m l i ol ine ee s C. ic i an e so e an e i ol ine ee o an ina an s o e a e esence of ne o eins in es onse o ea s oc as iffe en among iffe en iss es an a o m l i ol ine an i ol ine sil o ms es on e o ea s oc as e i ence e esence of a i ional o eins. as o e a e ession of ea s oc o eins in sil o m mig a in iffe en e el men al s ages ase on e e imen s sing S S A elec o esis 7 . i *et al.* anal e e e ession of e small ea s oc gene m S . in sil o ms C an fo n a ing le els of is o ein in iss es. as mos a n an in es is o a sil glan an ae. Song *et al.* fo n a e ea s oc 7 a o ein cogna e as one of e eg la e emoc ic o eins en sil o m la ae e on e o e inoc la ion of ea inac i a e ac e ia *Bacillus megaterium* .

S c al o eomics ma s o e s c e of o ein com le es o e o eins esen ing in a ecific cell la o ganelle f nc ional o eomics is a oa e m fo man ecific i ec e o eomics oac es an e ession o eomics is e an i a i e s of o ein e ession in sam les a iffe in some a ia le . e e a e een no lica ions o a e o ing e o eomics a oac o ea s oc o eins in sil o m. n e esen o o eome a e ns of fa o f om ea s oc e sil o ms as com a e i con ol sil o ms in o esis an an s s ce i le ee s an e ession a e ns of e iffe en ial o ein e e a ge e fo i en ifica ion. e fa o iss e of insec s a omolog e of mammalian li e as im o an f nc ions as a s o age iss e an as a e cen e of me a o lism an ioc emis .

MATERIALS AND METHODS

e ic eri s d k orm e ri

o ee s of sil o m *B. mori* . e ea ole an is a i a m l i ol ine ee an e ea s sce i le ing song a i ol ine ee f om C ina e e selec e ase on e io s e al a ions fo ea ole ance e Se ic l al esea c Cen e of C ina. o ee s e o i e e Sil o m ene ics an ee ing a o a o College of Animal Sciences e iang Uni e si . is a i is f om i cal egions e e e fiel em e a es of en eac es °C o ig e in s mme an i as e i i e ig ole ance o ig em e a es .

e imen al sil o m la ae e e ea e sing s an a ec ni es an con i ions in a 7. o com a e e effec of e os e o ele a e em e a e on males an fe males in en en l se i en ifica ion as ca ie o o se ing sil o m la al se ma s imaginal s on os e io a ominal sec ion of sil o m la a efo e ea s oc . l i ol ine females m l i ol ine males i ol ine females an i ol ine males e e se in e esen e e imen . o e al a e s i al a e of e ea e ose sil o ms e a la ae e e e ose o ea in eac ea men an en a no mal ea ing con i ion. e amo n an a ion of e os e in o e e imen e e i e same as i *et al.* an o an ina an .

erm re me s d m i

eca se e fa o is loca e n e e c icle in la ae ea s ess can easil eac e iss e en e la a is e ose o ea . On e fo a of e fif ins a sil o m la ae of eac gen e e e e ose o ei e °C fo min o °C fo in con olle g o c am e s. Af e e ea e os e e sil o ms e e e ne o e s an a ea ing em e a e °C an allo e o eco e . e fa o as emo e af e ea e os e an lace in ice col insec siological sal sol ion .7 aCl 7 . ee fa o sam les e e oole o minimi e a ia ion an o ge eno g iss e fo anal sis. a e as emo e f om sam les s o ime cen if ga ion. Con ol sam les of e fa o e e a e f om la ae a e e no e ose o ea . All la ae e e gene icall simila f om a single mo famil . All sam les e e s o e a 7 °C n il anal sis.

ro ei r c io d ec ro oresis

A mg sam le of fa o as omogeni e g in ing i in µ l sis sol ion 7 ea io ea C A S an m S . e sam le mi e as o e e an en inc a e fo min in ice. Si m i io ei ol an ffe ange as en a e . Af e cen if ga ion min a g °C e sol le o ein f ac ion as emo e an e o ein con cen a ion as e e mine sing e a fo me o . soelec ic foc sing as ca ie o i µg of o ein sam le in µ sol ion ea C A S /m eS ea ™ eagen an . ffe . o ein as loa e on o S s ange e in gel e a ion me o an s ec e o elec o esis sing an an o ni Ame s am a macia io ec a fo fo fo fo an fo . Af e se a a ion e s s e e imme ia el e ili a e × min in m is Cl ffe . con aining ea S S an gl ce ol. o e sam le i o e c ion an al la ion as a e in e fis e ili a ion s an . io oace ami e as a e in e secon e ili a ion s . e s s e e s ec e o e secon imensional elec o esis sing an an A si m l i le gel elec o esis ni eal ca e on of . ol ac lami e gels fo S S A . e elec o ese o eins e e s aine i a sil e s ain. ig gel lica es of eac ee ea e ose g o an con ol g o e e ea e ice.

eS ea ™ eagen ffe s an S s e e c ase f om eal ca e io sciences A S e en C A S an e e c ase f om US co o a ion Cana a io oace ami e as c ase f om eal ca e c ing ams i e U an ea an io ea e e c ase f om Ames am iosciences U an Sigma es ec i el . lec o esis e e c ase f om Am esco O US . eioni e a e ill o e ance i esis ance of . Ω cm as se o g o .

m e c isi io sis d ro ei de i ic io

o s e e scanne sing a ig esol ion image scanne Ame s am ioscience i els/gel an anal e mage as e sof a e e sion . olec la mass an *I* e e calc la e f om igi i e images sing s an a molec la mass ma e o eins. ac selec e o ic me e c i e ion a i as e ea e l esen in o gels as com a e in o ea men s an se es. n o e o meas e o ein e ession le els e s o ol me as cal c la e as a e cen age ela i e o e o al ol me of all e o s in e gel as no mali e a a o an if gel o s an se o e al a e o ein e ession iffe ences e een gels. o mali e ol mes of some o s e e anal e sing anal sis of a iance A O A S SS sof a e i ee fac o s incl ing e mal ea men ee an se .

o ein sam les e e is aine an sin iges e an e i es e e e ac e as esc i e else e e . S an S/ S ec a e e o aine sing e A 7 o eomics Anal e A O / O mass ec ome e A lie ios s ems . o eins e e anal e sing S/ S o anal sis an e e i en ifie i e a a ase sea c og am ASCO aemon a i Science agains C n /S iss o a a ase sing e follo ing a ame e s: en me sin fi e mo ifica ion ca ami ome l C . a ia le mo ifica ions o i a ion no es ic ion on o ein mass one misse clea age e i e c a ge + monoiso ic a e i e mass ole ance of m. o ein i en ifi ca ion i a confi ence in e al C. . o ein sco e g ea e an < as acce e in o S/ S an es l s. iological an molec la f nc ions e e fo n sing Uni o no le ge ase S iss o an ://.e as .o g/ o .

RESULTS

i i e om riso o ro ei ers

Com a ison of e fa o o eomes of o se es of ea e ose m l i ol ine an i ol ine ee s of sil o m an con ols is s o n in ig. . n ese o eome ofiles an 7 o s e e e ec e in i ol ine females an males es ec i el an an o s e e e ec e in m l i ol ine females an males es ec i el igi al im age anal sis an sing e same e ec ion a ame e s Smoo in A ea an Salienc . e n m e of o s as ig e in m l i ol ine sil o ms an in i ol ine sil o ms an as ig e in males an in females a le . ig. s o s iffe en iall e esse o ein o s in i ol ine sil o ms an iffe en iall e esse o ein o s in m l i ol ine sil o ms in es onse o ea e os e. e e a e o egions in e gels ic s o al e e e ession of o eins in o m l i ol ine an i ol ine sil o ms ea s oc a e ns. ese a e n m e e o egion an o egion in ig. . ese e esse o s a e e simila fo o ee s an gi e o ci le s aining a e ns common es onse o s . Al o g o ein o is i ion a e ns iffe e een e o ee s e a e simila i in eac ee e een e se es an ea ea men s an °C . e efo e se an e o ea ea men s can e oole fo is e e imen . esi es e common es onse o s o s e e a e o s o s in e i ol ine sil o ms an o s o s in m l i ol ine sil o ms in ce ea s oc an

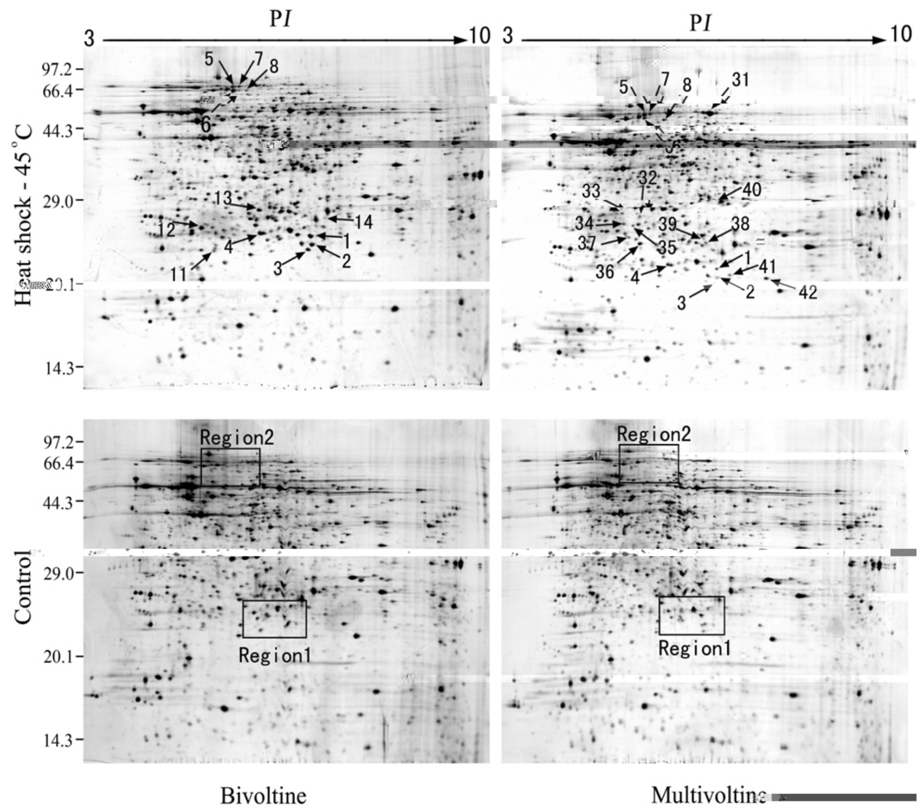

Fig. 1. 2D electrophoresis protein profiles of fat body of the control and heat exposed silkworm larvae from the thermo-susceptible

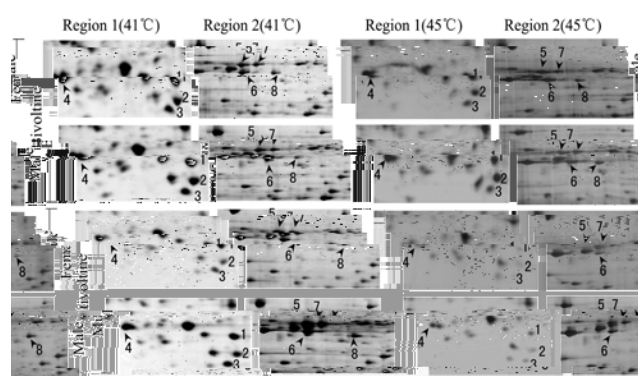

Table 1. List of identified silkworm fat body proteins in responses to high heat exposure

Spot no.	Protein name (Matched organism)	Accession GI no.	No. of peptides (coverage)	Protein score (C.I. %)	*Mr* calcd/obsd (P*I* calcd/obsd)	Ontology
	Common response spots					
1	Heat shock protein HSP20.4 (*Bombyx mori*)	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 (*Bombyx mori*)	56378317	7 (29.24%)	120 (100)	24 / 19.88 (6.23 / 6.53)	Response to stress
4	Heat shock protein HSP20.8 (*Bombyx mori*)	11120618	7 (46.09%)	177 (100)	26 / 20.79 (5. 80 / 5.98)	Response to stress
5	Heat shock protein HSP70 (*Antheraea yamamai*)	47232576	16 (40.28%)	98 (99.96)	85 / 69.55 (5.80 / 5.7)	Response to stress ATP binding
6	Heat shock protein HSP70 (*Antheraea yamamai*)	47232576	12 (25.87%)	110 (99.98)	80 / 69.55 (5.9 / 5.7)	Response to stress ATP binding
7	Heat shock protein HSP70 (*Antheraea yamamai*)	47232576	13 (29.65%)	102 (99.98)	85 / 69.55 (5.9 / 5.7)	Response to stress ATP binding
8	Heat shock protein HSP70 (*Antheraea yamamai*)	47232576	15 (45.40%)	86 (99.44)	79 / 69.55 (6.15 / 5.7)	Response to stress ATP binding
	Specific response spots (Bivoltine)					
11	Heat shock protein HSP20.1* (*Bombyx mori*)	112983134	7 (33.00%)	84 (97.80)	25 / 20.18 (5.51 / 5.46)	Response to stress
13	PRETICTED: similar to zinc finger protein 436 (*Canis familiaris*)	57048379	7 (20.54%)	80 (97.74)	30 / 55.30 (6.3 / 8.94)	Zinc ion binding
	Specific response spots (Multivoltine)					
34	PREDICTED: similar to CG10504-PA* (*Tribolium castaneum*)	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 (*Bombyx mori*)	56378321	8 (60.43%)	120 (100)	29/2139 (5.74 / 5.79)	Response to stress
38	PREDICTED: similar to zinc finger protein 46* (*Canis familiaris*)	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* (*Apis mellifera*)	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.

ge as e sof a e. ese anal ses e eale a e es sion insensi of some s o s e e iffe en in m l i ol ine an i ol ine ee s. Among e i en ifie o eins of e i ol ine ee s o s]] an] s o e a s ecific eg la ion n e o ea e os e ea men s. Among e] i en ifie o eins of m l i ol ine ee s o s an e e eg la e in es onse o o ea e os e ea men an s o s an] s o e eg la ion in a] °C ea men alone. ese iffe ences s o e a in e m l i ol ine ee e n m e of e esse o ein s o s inc eases in es onse o an inc ease in e ea e os e em e a e o e e in e i ol ine ee no iffe ences e e o se e e een e ea e os e ea men s. s o 7 as eg la e onl in females of e m l i ol ine ee . e e a e iffe ences in e ol me of o ein e ession e een i ol ine an m l

i ol ine ee s in o ea e os e ea men s as ell as e een se es. a le] esen s e means of e no mali e ol mes ol me e cen age of common es onse s o s incl ing s S s egion] an S 7 egion se a a e ea men ee an se . Significan iffe ences e een e o ees an e em e a e e os es e e e e mine A O A a le . a a in a le] an A O A in a le e eale a common es onse o ein s o s e e e esse in eac of e sam les. o e e e an i of S s iffe e . o eo e o ein e ession in ensi of s S s egion] iffe e significan l <] e een e o ea e os e ea men s an e sil o m ee s. e e ession of s S s in e m l i ol ine ee as lo e an in e i ol ine ee af e e °C ea e os e ea men ile e e as no significan iffe ence

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 e intensi of oein e ession e een ee s af e e °C ea e os e ea men . n o e o s a lo e ea e os e ea men s sil o m ees i no iffe significan l in ei es onse ile a ig e em e a e e os e ea men s e e mo ole an ee e esse significan l lo e s S s < . e iffe ences e een e o em e a es e e no significan fo S 70 in e m l i ol ine ee . o e e ession of s S s an s a le e ession of S 70 s gges e a S 70 an s S ma la iffe en ole in e mal ole ance a ig e em e a e in e e mo ole an ee . Com a ison of e ol me e een e o se es in ica es a e e a e some iffe ences in oein e ession al o g i as no signifi can a le .

DISCUSSION

e mal sensi i i an ea s oc es onse of iffe en aces of *B. mori* can e meas e o se ing e s i al a e of la a a mo an egg an o se ing cocoon c a ac e is ics . e s of e ea s oc es onse on e molec la le el gi es mo e info ma ion a o ea s oc oeins an ioma e s. en ifica ion of oein ma e s ill also o i e ee e s i a mean fo mo e effi cien an co ec selec ion of ea ole an ai s 7 . e i en ifie oeins a a e iffe en iall e esse af e ea e os e of ic a e no n S s an a e e ice o e in ol e in ea s oc es onses. e me o s e se in ese e e imen s incl ing ig esol ion gel elec o oesis of fa o sing sil e s aining com ine i S/ S an S anal sis of mass ec ome o e o e a s ccessf l s a eg in e s of S s in iffe en sil o m a ie ies. e c anges in oein e ession as a es l of ea s oc es onse e e no i en ical in e o ee s. is s g ges s some clea can i a e ma e oeins fo i en if ing ea ole an an ea s sce i le sil o m la ae. ene all e m l i ol ine ee as s o n ig e s i al a es an e i ol ine ee in es onse o ea s oc . o n in a *et al.* s o e a e is a i ee ic is e m l i ol ine ee se in e esen e e imen is e mos ole an ee among m l i ol ine ee s. ecific es onse oeins incl ing o s an ma se e as ma e oeins fo ea s sce i le an ea ole an es ec i el . n a ic la oein o s an in i ol ine ee an o s an in m l i ol ine ee can e consi e e as oein ma e s ela e o ole ance. S las *et al.* 7 o se e a 7 oein o s e e e esse in a ea s oc ole an c l i a of ea af e ea s oc sing o eome anal sis. S le *et al.* also se o eomic anal sis A S o e ec e effec s of ea s oc on an a io ic s ess ole an an an a io ic s ess s sce i le c l i a of a le . e fo n o oeins o s ni e o e s ess s sce i le c l i a .

n is o e i en ifie lo molec la eig S oeins . . . an . ic e e e esse af e ea e os e. Sa ano *et al.* e o e a *B. mori* a si s S s incl ing e a o e esc i e s S s an s S .7 . S las *et al.* 7 e o e a e ma o i of e ea s oc oeins in o e ea s sce i le an e ea ole an c l i a s of ea a lo molec la eig . o oein o s in egion of o ee s e e S 70. nc eases in S 70 can o ec in ac la ae agains e e mal inac i a ion of alco ol e ogenase an agains e mal in i i ion of fee ing . S 70 la s a cen al ole in s ess ole ance incl ing omo ing g o a mo e a el ig em e a es an o ec ing o ganisms f om mo ali a e eme em e a es c a e oning nfol e oeins . Once fol e e l ese oeins a e less sensi i e o ena a ion an agg ega ion. ee e esse oeins ic a e simila o inc finge oein i en ifie in is esea c a e li el in ol e in e fol ing ocess of oeins ecause inc finge s a e in ol e in fol ing of o eins.

e e ession of s S s in e m l i ol ine ee is significan l < lo e an in e i ol ine ee en e ose o e °C ea men e e is no iffe ence e een e ees en e ose o e °C ea men a le an ig. . is emons a es a e e mo ole an sil o m ee as no c a ac e i e a ig e le el of s S s n esis n e se e e ea s oc as com a e o e e mosensi i e ee . S e ession as no significan l e ce a e ig e em 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 ms. ase 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 ecific o eins in ol e in e ole ance of m l i ol ine ee mig also a e an im o an ole as e a e ec e s o s in e m l i ol ine ee com a e i s o s in e i ol ine ee en e ose o ea . S ilo a *et al.* 7 concl e a e mo ole ance e i e se e al al e na 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 io s esea c as s o n a in e mo ole an ees 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 Af ica as a lo e le el of S s n esis n e mo e a e ea e os e 7. °C com a e o e less e mo ole an O egon s ain 7 .

ig. an a le s o a male sil o m la a e esse slig l mo e S eciall in e m l i ol ine ee e iffe ence is no significan < . e n m e of o ein s o s e ec e image anal sis sof a e is also ig e in males an in females. o e es of o no le ge no o e lica ion isc sses e iffe ences e een female an male sil o ms 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 se es of sil o m la ae.

n o e o i en if mo e o ein ma e s an o en ance o n e s an ing of e ela ions e een sil o m ees s an ei iffe en e mal ole ances an ei e essions of iffe en 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 ees. A i ional me o s fo e o e sil o m iss es s o l also e e lo e . n e f e e efo e e ill in es iga e e effec s of e cessi e ea s oc on e o eome of iffe en ees s an se es.

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 ogeno s genes en enginee ing ansgenic sil o m . e efo e ma m 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 ic la l in e n e s an ing e iffe en ial e ession a e n of a io s S s in i ol ine an m l i ol ine ees. e im o ance of S ic as confi me fo sil o m la ae e mo ole ance in e esen esea c o l g ea l facili a e is esea c .

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