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

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¹ College of Animal Sciences ² liang Uni e is $f = 4 \operatorname{Ing}^{p} \operatorname{o}_{k} \circ 100^{2}$. \widehat{C}^{1} ina ² Ag ict_k l t e Fac_k l t Uni e si t of t t lan Pas² an ³ Se ict_k l t al Pesea \widehat{C}^{1} ns i t e ³ liang Aca em_y of Ag ict_k t t al Sciences ³ ang² ot_k $\circ 100^{2}$ 1 \widehat{C}^{1} 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 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. © KSBB

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

INTRODUCTION

 T^{i} e se icult e in us T^{i} as con i_{01}^{i} e significanly o T^{i} e sconomic e elormen of many coun ies. T^{i} is T^{i} as e sule in en ic e sil wom ge molasm esources the o esources is e o esources in generics an T^{i} e so f sil wom T^{i} e esource of generics an T^{i} e so f sil wom T^{i} e esource of generics an T^{i} e so f sil wom T^{i} esource of a esource of generics an T^{i} esource of a source of generics an esource of generics an T^{i} esource of a source of generics an esource of generics an T^{i} esource of a council esource of generics an T^{i} esource of a council esource of generics of generics an T^{i} esource of a council esource of generics of generics of generics of generics of generics an T^{i} esource of a council esource of generics of

Corresponding author Tel: +86-571-86971657 Fax: +86-571-86971657 e-mail: chenyy@z ge gcn ion \hat{I} e mal ole ance an isease esis ance 1. $T\hat{I}$ e mo ole ance is value in the selection of silve in silve models in silve in the selection of silve models in silve in the selection of silve models in the selection of silve models in the selection of silve models is selected in the selection of silve models in the selectin the selectin the selection of silve models in the selection of

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Such al o ecomics mays our fees us the of o ein completes of ferror eins essening in a specific cellula o ganelle functional o ecomics is a 'o oal elm formany specific i ecler o ecomics and oac' est an elession o ecomics is ferror and a sector elevent and the elevent no withications of a elevent ing fee o ecomics and oac' of east oclere o ecomics ing ferror ecomics and oac' o ecome values for a elevent ing ferror ecomics and oac' o ecome values of a 'ferror ecomic ecomics and oac' o ecome values of a 'for form ferror ecomics and oac' o ecome value entropy form ferror ecomics and oac' o ecome value entropy form ferror ecomics and oac' o ecome value entropy form ferror ecomics and ecomic ecomic elevent ecomic ecome ecomic ecomic ecomic ecomic ecome ecomic ecomic

MATERIALS AND METHODS

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Two 'o ee s of sil wo m (B. mori .) $\hat{I} e^{\hat{I}}$ ea ole an is a i (a mul i ol ine 'o ee) an $\hat{I} e^{\hat{I}}$ ea susce i 'd e ing song (a 'd ol ine 'o ee f om $\hat{C}^{\hat{I}}$ ina) we e selece 'ase on • e ious e alua ions fo \hat{I} ea ole ance 'o, \hat{I} e Se icul al • esea c' Cen e of $\hat{C}^{\hat{I}}$ ina. $\hat{O}^{\hat{I}}$ 'o ee s we'e • o i e 'o, \hat{I} e Sil wo m ene ics an ee ing a 'o a o, College of Animal Sciences \hat{I} biang Uni e si, is a i'is f om ovi cal egions \hat{J} e fiel emve a t es of en eac' es 4CC $\hat{O}^{\hat{I}}$ ig' e in summe an i \hat{I} as $e^{\hat{I}}$ i'd e \hat{I} ig' ole ance o ig' emve a t es I'.

we imen al sil voom la aevee e ae using san a ec'ni us an con i ions in a 2007. To comvae 'e effec of e vost e o ele ae emve au e on males an fe males in even en ly se i en ifica ion vas ca ie ou 'oy o'se ing sil voom la al se ma s (imaginal 'di son vos' e io a'o ominal sec ion of sil voom la a') 'efo e ' ea s' oc . ul i ol ine females mul i ol ine males 'di ol ine females an 'di ol ine males vee use in 'e vesen e ve imen. To e alua e su i al ae of 'e ai neac' ea men an 'en e a no mal ea ing con i ion. T' e amoun an u a ion of e vost e in ou e ve imen vee vi² 'e same as i *et al.* 1 an o van ovina 'an 1'.

7 erm יTre me⊸_is ⊶d Asm יi⊸_i

ecause i e fa 'o , is loca e un e i e cu icle in la ae i ea s ess can easil, 'eac' i e issue v en i e la a is e vose o i ea. On i e fou i a, of i e fif i insa \sim sil vom la ae of eac' gen e ve é e vose o ei i e 45° C fo 75 min o 4PC fo 1° in con olle govi c am'res. Af e i e i ea e vosu e i e sil voms ve e e une o i e san a ea ing emve a u e (24C) an allove o eco e . T° e fa 'o y was emo e 2^{3} af e ⁷ ea e vost e an vlace in ice col insec 1^{3} siological sal solt ion (C7 aCl) 17. T¹ ee fa 'o y samules we e voole o minimire a ia ion an o ge enoug¹ issue fo analysis. Wa e was emo e f om samules 'oy s¹ o ime cen if tga ion. Con ol samules of ³ e fa 'o y we e vera e f om la ae ³ a we e no e vose o ³ ea. All la ae we e gene ically simila (f om a single mo³ family). All samules we e s o e a 72° C tn il analysis.

,, roei→, rcio→, →d vecrooresis

A $^{\circ}$ Cmg samule of fa $_{00}$, was 1 omogenize $_{00}$ g in ing i in 45 Cµ l sis solution (7 t ea 2 1 iou ea 4 C TArS an 1 m $^{\circ}$ SF). T e samule mi t e was o e e an ¹ en inc₄ a e fo 10 min in ice. Si m i¹ io ¹ ei ol (DTT) an 2^{n} ouffe (-1 ange 1 10 was ¹ en a e. Af e cen ifuga ion (? ()min a B ()(0) g 4C) ¹ e sol_{u} de \bullet o ein f ac ion was emo e an ${}^{\mathcal{I}}e \bullet$ o ein con cen a ion was e e mine $\frac{1}{2}$ sing $\frac{1}{2}$ e a fo me $\frac{1}{2}$ o $\frac{1}{2}$ 1. soelec ic focusing was called out $ui^{\mathcal{I}}$ $0 \mu g$ of \bullet o ein samule in $\pounds 0 \mu$ solution (/m DeS ea TM Deagen an 0.5 ^P 'ouffe \bullet ^{TP} 10. P o ein was loa e on o ^P D S iss (\bullet ^T ange 2 10 'ov The ingel e^2 , a ion me² o and subject to elec or of of esistisming and an r^3 o $F_{1,11}$ (Ame s² am r^3 a macia io ec²) a r^2 of 2^{-3} 500 fo 1^{-3} 2000 fo 1^{-3} 4 CCC fo $1^{\hat{i}}$ an CCC fo $1C^{\hat{i}}$. Af e F set a a ion Tes is we e imme ia el, e tilio a e 2×5 min in 50m T is TC offe (1,) con aining t ea 2 SDS an ° 0 gl ce ol. Fo [°] e samule wi[°] o₄ e 4c ion an al la ion $\mathbf{D}TT(1)$ was a e in ^Te fis e uilio a ion s e in 2.5 io oace ami e was a e in 2 e secon e uili a ion s e T^{\dagger} e s i s we e subject e o T^{\dagger} e secon imensional elec of o esis using an an DA Tsi multi le gel elec of o esis uni ($Teal^{4}$ ca e) on o o of 2.5 \bullet ac lami e gels fo SDS $\cap A$. T e elec of o ese \bullet o'eins we e s aine wi a sil e s ain. ig gel e laca es of eac' bee ('ea e vose $g o_k \bullet an$ con ol $g o_k \bullet$) we e e vea e vuice.

DeS ea TM Deagen n' ouffe s an n' D. S is sue e a c' ase f om "Eal¹ ca e io sciences A' (Sue en) C'TA'S an DTT use e a c' ase f om US co to a ion (Cana a) io oace ami e was a c' ase f om "Eal¹ ca e (tc ing¹ ams¹ i e U) an t ea an ¹ iot ea we e a c' ase f om Ames¹ am iosciences (U) an Sigma estec i el, lec o o esis we e a c' ase f om Am esco (O'T US). Deionife wa e (illigo e F ance) wi² esis ance of 1.2 Ω cm was use ¹ oug² ot .

ˈm e carisiio⊸ı ⊸ıısis ⊸d_uroei⊸ı ˈde⊸ıiic io⊸ı

Sub s we e scanne $t_s \sin g a^2 i g^2 = \operatorname{sol}_{t_1} \operatorname{ion} \operatorname{image scanne}$ (Ame s² am ioscience ° () ui els/gel) an anal y e 'oy mage as e 2D sof wa e (e sion °). olect la mass an u we e calcula e f om igi i e 2D images t sing s an a molect la mass ma e $\circ o$ eins. ac selece sub w ic me \hat{i} e c i e ion \hat{i} a i was even e l, esen in wo gels was comma e in $\hat{i}_0 \hat{i}$ ea mens an se es. no e o meast e o ein e ession le els \hat{i} e suo olume was cal cula e as a e cen age ela i e o \hat{i} e o al olume of all \hat{i} e suo s in \hat{i} e gel as no malife a a o un if, gel suo s an use o e alua e o ein e ession iffe ences \hat{i} e ween gels. o malife olumes of some suo s we e anal fe using anal sis of a iance (A \bullet A) \hat{i}_{0y} SrSS sof wa e wi \hat{i} d e fac o's incluing \hat{i} e mal e a men \hat{i}_0 ee an se.

Po ein samules we e is aine an usin iges e an evi es we e e ac e as esc i'e elsevie e 22. S an S/S succ a we e o'o aine using ${}^{7}e A = 4700$ Po eomics Anal Fe A D TOF/TOF mass succ ome e (Avalie ios's ems). Po eins we e anal Fe using S/S o P F anal sis an we e i en ifie vi ${}^{7}e = a a'_{0}ase sea c'$ og am 'ASCOT Daemon (a i Science) agains C n/Swiss • o a a'o ase using ${}^{7}e$ following a ame e si enfume usin fi e mo ifica ion ca 'o ami ome ${}^{7}l (C)$ a ia'd e mo ifica ions o i a ion () no es ic ion on • o ein mass one misse clea age ve i e c' a ge + 1 monoiso ovic a ve i e mass ole ance of 100 vem. Po ein i en ifi ca ion vi a confi ence in e al (C.) • o ein sco e g ea e ${}^{7}an > 5$ (P < 0.5) was acceve in 'o ${}^{7}S/S$ an P F estils. iological an molecula funcions ve e foun 'ov using UniP o novale ge'o ase (Swiss P o an T) (${}^{6}v/V$ varaate vas vo g/s • o).

RESULTS

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Commu ison of \hat{i} e fa ' $_{00}$, \hat{j} o eomes of ' $_{00}$ \hat{i} se es of \hat{i} ea e sose multi ol ine an ' $_{0i}$ ol ine ' $_{0}$ ee s of sil wo m an con ols is si own in Fig. 1 n \hat{i} ese \bullet o eome \bullet ofiles 5° 4 an 7 44 sub s vie e e e e e in $\frac{1}{2}$ i ol ine females an males estec i el, an 5 2 an 25 sub s vie e e e e e in m_{ij} l i ol ine females an males estec i el y oy igi al im age anal sis an using 1 e same e e c ion ${}^{\prime}$ a ame e s (Smoo 1 2 in A ea 5 an Salienc, 1 1 0 1 e num e of $s = 0 s = as^{T} ig^{T} e$ in $m_{th} [i ol ine sil] = 0 ms^{T} an in] i ol ine$ sil wo ms an was ig e in males an in females (Table 2). Fig. $1s^7$ ous 2 iffe en iall r e \bullet esse \bullet o ein s \bullet o s in $\frac{1}{10}$ ol ine sil $\frac{1}{10}$ oms an 20 iffé en iall, e $\frac{1}{10}$ esse $\frac{1}{10}$ o ein so s in multiplication in the contained of the contained (egion 1) an $5 \circ (\text{egion } 2)$ in Fig. 2. T^f ese $e \bullet esse$ sos a e e simila fo 'o^T 'ee san gi e eo uci'de s aining wa e'ns (common eswonse swo s). Al $\sigma_{kg} q^{l} \bullet o$ ein sto is i'd ion ta ens iffe 'e ween 'e wo bees ¹e, a e simila wi¹ in eac¹ bee be ween ¹e se es an ¹ea ea men s (45 an 4PC). T¹e efo e se an ¹e wo¹ea ea men s can 'e voole fo ¹ is e ve imen. esi es ¹ e common esymonse suo s (suo s 1) 1 e e a e 4 suo s (suo s 11 14, in ¹ e 'd ol ine sil wo ms an **2** soos (soos $1 \stackrel{(1)}{\cancel{2}}$ in m₄ l i ol ine sil wo ms in $\frac{1}{\cancel{2}}$ ce $\frac{1}{\cancel{2}}$ ea s⁷ oc an



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



Spot no.	Protein name (Matched organism)	Accession GI no.	No. of peptides (coverage)	Protein score (C.I. %)	<i>Mr</i> calcd/obsd (P/ calcd/obsd)	Ontology
	Common response spots					
1	Heat shock protein HSP20.4	49036077	9	148	26/20.41	Response to stress
	(Bombyx mori)		(49.86%)	(100)	(7.10/6.54)	
2	DNA-formamidopyrimidine glycosylase*	163793016	7	90	25/32.87	Zinc ion binding, DNA
	(alpha proteobacterium BAL199)		(31.00%)	(99.42)	(7.05 / 8.57)	binding; catalytic activity
3	Heat shock protein HSP 19.9	56378317	7	120	24/19.88	Response to stress
	(Bombyx mori)		(29.24%)	(100)	(6.23 / 6.53)	
4	Heat shock protein HSP20.8	11120618	7	177	26/20.79	Response to stress
	(Bombyx mori)		(46.09%)	(100)	(5. 80 / 5.98)	
5	Heat shock protein HSP70	47232576	16	98	85 / 69.55	Response to stress
	(Antheraea yamamai)		(40.28%)	(99.96)	(5.80 / 5.7)	ATP binding
6	Heat shock protein HSP70	47232576	12	110	80/69.55	Response to stress
	(Antheraea yamamai)		(25.87%)	(99.98)	(5.9 / 5.7)	ATP binding
7	Heat shock protein HSP70	47232576	13	102	85 / 69.55	Response to stress
	(Antheraea yamamai)		(29.65%)	(99.98)	(5.9 / 5.7)	ATP binding
8	Heat shock protein HSP70	47232576	15	86	79 / 69.55	Response to stress
	(Antheraea yamamai)		(45.40%)	(99.44)	(6.15 / 5.7)	ATP binding
	Specific response spots (Bivoltine)					
11	Heat shock protein HSP20.1*	112983134	7	84	25/20.18	Response to stress
	(Bombyx mori)		(33.00%)	(97.80)	(5.51 / 5.46)	
13	PRETICTED: similar to zinc finger	57048379	7	80	30 / 55.30	Zinc ion binding
	protein 436 (Canis familiaris)		(20.54%)	(97.74)	(6.3 / 8.94)	
	Specific response spots (Multivoltine)					
34	PREDICTED: similar to	91079909	14	82	33/51.45	Transferase activity;
	CG10504-PA* (Tribolium castaneum)		(33.00%)	(96.20)	(5.45 / 7.77)	protein amino acid
			. ,	. ,	. ,	phosphorylation
36	Heat shock protein HSP21.4	56378321	8	120	29/2139	Response to stress
	(Bombyx mori)		(60.43%)	(100)	(5.74 / 5.79)	
38	PREDICTED: similar to zinc finger	57048379	13	96	29/55.30	Zinc ion binding
	protein 46* (<i>Canis familiaris</i>)		(27.00%)	(99.84)	(6.91 / 8.94)	Ŭ
40	PREDICTED: similar to CG9935-PA	66507549	11	86	37/61.90	Transferase activity:
	isoform 1* (Apis mellifera)	2000.010	(27.00 %)	(98.50)	(7.04 / 6.14)	protein amino acid
			(()	(phosphondation

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

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

*Identification of protein by PMF analysis.

ge as e 2 sof va e. T^{i} ese anal ses e eale T^{i} a e \bullet es sion in ensi , of some seo s ve e iffe en in multi ol ine an 'j ol ine 'j es s. Among T^{i} e 4 i en ifie \bullet o eins of T^{i} e 'j ol ine 'j es seo s 11 an 2 sⁱ ove a secific to egula ion un e 'j $0^{T^{i}}$ ea e vost e ea men s. Among T^{i} e 2i en ifie \bullet o eins of multi ol ine 'j es seo s 4Can 2 ve e to egula e in esconse o 'j $0^{T^{i}}$ ea e vost e ea men an seo s T^{i} an 41 sⁱ ove to egula ion in a 4PC ea men alone. T^{i} ese iffe ences sⁱ ove T^{i} a in T^{i} emulti ol ine 'j ee T^{i} entre of e \bullet esse \bullet o ein so s inc eases in esconse o an inc ease in T^{i} ea e vost e emute at e T^{i} ove e in T^{i} e 'j ol ine 'j ee no iffe ences ve e o'j se e 'j e veen T^{i} e a e vost e ea men s. Suo T^{i} vas to egula e only in females of T^{i} emulti ol ine 'j ee T^{i} e e a e iffe ences in T^{i} e oltime of \bullet o ein e \bullet ession 'j e veen 'j ol ine an multi i ol ine 'oee s in 'o² i ea e vost e ea men s as vell as 'oe veen se es. Ta'de 1 vesen s i e means of i e no malife oltimes (oltime ve cen age) of common esconse soos incluing 4s Sr's (egion 1) an 4 Sr'7 ((egion 2) seva a e 'oy ea men 'oee an se. Significan iffe ences 'oe veen i e voo'oee s an i e emve at e e vost es ve e e e mine 'oy A • A (Ta'de 2). Da a in Ta'de 1 an A • A in Ta'de 2 e eale i a common esconse • o ein s to s we e e vesse in eaci of i e samules. To ve e i e tan i of Sr's (egion 1) iffe e significan 1. (r) < 0.01) 'o veen i e voi ea e vost e ea men s an i e sil voo m 'oee s. Ti e e vession of s Sr's in i e multi ol ine 'oee vas love i an in i e 'j ol ine 'oee af e i e 45°C ea e vost e ea men vi ile i e was no significan iffe ence

Dreed	Sex	Heat treatment (45°C)			Heat treatment (41°C)	
Breed		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)

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

*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)

	-					
Sourco	df -	sHSP		HSP70		
Source		M.S.	Р	M.S.	Р	
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 7 e in ensi, of \bullet o ein e \bullet ession 'e veen 'o ee s af e 7 e 4PC 7 ea 'e vost e ea men. n o e vo s a love i ea e vost e ea men s sil vo m 'o ee s i no iffe significan l, in i ei es vonse vi ile a i g e em ve at e e vost e 'ea men s i e e mole an 'o ee e \bullet esse significan l, love s 7 B'rs (r < C C). T^{4} e iffe ences 'e ween i e vo em ve at es ve e no significan fo 7 B'r7 C in i e mul i ol ine 'o ee . ove e \bullet ession of s B'r7 C may vlay iffe en ole in i e mal ole ance a i g e em va at e in i e i e mole an 'o ee . Conva ison of i e ol ume 'e veen i e vo se es in ica es i a i e e a e some iffe ences in \bullet o ein e \bullet ession al ot g i i vas no signific can (Ta'de 2).

DISCUSSION

Tⁱ e mal sensi i i , an ⁱ ea sⁱ oc es conse of iffe en aces of *B. mori* can 'e measu e 'o, o'se ing ⁱ e su i al a e of la a ca moⁱ an egg an 'o, o'se ing cocoon cⁱ a ace is ics $1^{i} 2^{i} 2^{i}$. If ⁱ e su of ⁱ e ⁱ e a sⁱ oc es conse on ⁱ e molecula le el gi es moⁱ e info ma ion a'ou ⁱ ea sⁱ oc • o eins an 'joma e s. en ifica ion of • o ein ma e s will also • o i e 'o ee e s wiⁱ a mean fo mo e effi cien an co ec selec ion of ⁱ ea ole an ai s 27. We i en ifie 14 • o eins ⁱ a a e iffe en iall, e esse af e ⁱ ea e vost e of vici a e noun "S's an 5 a e • e ice o 'e in ol e in ⁱ ea sⁱ oc es conses. Tⁱ e meⁱ o s we tse in ⁱ ese e • e imen s inclu ing ⁱ igⁱ esolu ion 2D gel elec o

♦ o esis of fa '₁₀ , using sil e s aining com'₀ine wi¹ S/ S an S anal'₁sis of mass sweet ome , • o e o '₀e a successful s $a eg_{v}$ in $\overline{f} e s_{u}$ of $\overline{f} s$ in iffe en sil uo m a ie ies. $\overline{T}^{4} e c^{2}$ anges in $\bullet o e$ in $e \bullet e$ ssion as a $es_{u}l$ of $\overline{f} ea$ s^{T} oc es conse we e no i en ical in r^{T} e wo o ee s. T^{T} is sug ges s some clea can i a e ma e • o eins fo i en if ing rea ole an an rea susce•i'de sil wo m la ae. ene all remul i ol ine 'o ee ras s'own ig e su i al a es ran re i ol ine bee in estonse of east oc 12. oun inta et al. 2 s'oue a e isai oee wich is e mul i ol ine 'o ee use in 'e vesen e ve imen is 'e mos ole an 'pee among 11 multi ol ine 'pee s. Suecific es onse o eins incluing so s 11 14 an 21 2 ma se e as ma e • o eins fo ¹ ea susce•i'de an ¹ ea ole an' estec i el , n va icula vo ein stos 11 an Γ in \dot{b} o line be an $s \circ s \circ 4$ an 4 cin mul i ol ine be can be consi e e as \circ o ein ma e s ela e o ole ance. S clas *et al.* 27 obse e ¹ a 7 • o ein soos weee • esse in a¹ ea s'oc ole an culi a of wheat afe deated as octating to o eome anal sis. S le *et al.* 2_{12} also use \bullet o eomic anal sis S) o e ec \hat{i} e effects of \hat{i} ea \hat{s} oc on an 2D MA a jo ic s ess ole an an an a jo ic s ess suscevi de cul i a of bale, T'e, foun wo o eins so s uni le o 'e s ess suscevi'le cul'i a.

n⁴ is wo we i en ifie 5 low molecula weig¹ **S**ⁿ o eins (20,4 1... 20,4 20,1 an 214, wi ic¹ we e e esse af e⁻¹ ea e osu e. Sa ano *et al.* °0 eo e⁻¹ a *B. mori*¹ a si s^{*} **S**ⁿ s incluing⁻¹ e a'o e esci'e s^{*} **S**ⁿ s an s^{*} **S**ⁿ 2^{*}.7. S las *et al.* 27 evo e⁻¹ a⁻¹ e mino i of ¹ e¹ ea s¹ oc o eins in 'o⁻¹ e¹ e a suscevi'd e an 'i e ea ole an culi a s of we a⁻¹ a low molecula weig¹. Fou o ein so s in egion 2 of 'o⁻¹ e e s we e⁻¹ **S**ⁿ 70 nc eases in "**S**ⁿ 70 can o ec in ac la ae agains ¹ e ¹ e mal inaci a ion of alco¹ ol e¹ og no ing g ow¹ a mo e a el ¹ ig¹ en e a t es an o ec ing o ganisms f om mo ali a e eme em e a t es 'o c¹ are oning unfol e o eins ² . Once fol e o ore l, 'I ese o e eins a e less sensi i e o ena t aion an agg ega ion. T¹ e e e o esse o eins v¹ ic¹ a e li el, in ol e in ¹ e fol ing o cess of • o eins 'ecause Finc finge s a e in ol e in fol ing of • o eins.

T² e e ession of s **B**'s in ¹ e multi ol ine 'o ee is sig nifican l, (? < **C**, **D**) lowe ¹ an in ¹ e 'o ol ine 'o ee with en e tose o ¹ e 45°C ea men 'ot ¹ e e is no iffe ence 'e ween ¹ e 'o ee s with en e tose o ¹ e 4PC ea men (Ta'de 2 an Fig. 2). T² is emons a es ¹ a ¹ e ¹ e mo ole an sil wo m'o ee was no c¹ a ac e it e 'o, a¹ ig¹ e le el of s **B**'? s, n¹ esis un e se e e¹ ea s¹ oc 'as comva e o ¹ e ¹ e mosensi i e 'o ee . (p **B**'?? Ce to ession was no significan l, e uce a ¹ e ¹ ig¹ e em e a u e ea men . T¹ is sugges s ¹ e s **B**'? an **B**'?? Ona, ta, iffe en ole in ¹ e mo ole ance of sil wo ms. ase on ¹ e a aila'de esea c¹ a eat o s conclue ¹ a o¹ e mec² anisms mig¹ 'e in ol e wit¹ a emo ole ance o¹ e ¹ an ¹ e s **B**'? s an ¹ e **B**'?? C T³ e num'e of stecific to eins in ol e in ¹ e ole ance of multi ol ine 'o ee mig¹ also³ a e an into an ole as we¹ a ecce **2** sto s in ¹ e multi ol ine 'o ee comva e wi² 4 sto s in ¹ e 'j ol ine 'o ee with en e tose o ¹ ea. S¹ ilo a *et al.* 7 conclue ¹ a ¹ e mo ole ance e ti e se e al al e na i e molecula mec² anisms an ¹ a **B**'? 4C s **B**'s an o³ e uni en ifie fac o s ta e an im o an ole in ¹ is to cess along wit³ **B**'?7 C in *D. melanogaster.* ¹ e ious esea c¹ as s³ own ³ a in ³ e mo ole an 'o ee s of *D. melanogaster* **B**'?7 C's on ¹ esis is main aine a low le els. T³ e mos ¹ e mo ole an's a in T (isola e in Cen al Af ica) ¹ as a lowe le el of **B**'?7 C's on ¹ esis in e mo ea e¹ ea e ost e (?7.5°C') comva e 'o ¹ elses ¹ e mo ole an (2 egon **s** ain 7 2 1.

Fig. 2 an Ta'de 2 s⁷ ow ⁷ a male sil vom la a e esse slig⁷ ly mo e "577 0 estecially in ⁷ e multi ol ine'oee 'du ⁷ e iffe ence is no significan ($r^{0} < 0.01$). T⁷ e num'de of o ein stors e ece 'dy image analysis software is also ¹ ig⁷ e in males ⁷ an in females. To ⁷ e des of our novale ge no o⁷ e ou'dica ion iscusses ⁷ e iffe ences 'de ween female an male sil woms in eston ing o⁷ ea ole ance. Fu⁻⁷ e e te imen a ion is e ui e o e e mine ⁷ e iffe ence in ⁷ e mo ole ance 'de ween ⁷ e se es of sil wom la ae.

no e o i en if, mo e \bullet o ein ma e s an o en ance ou un e s an ing of \hat{i} e ela ions \hat{i} i be ueen sil uo m o e s an \hat{i} ei iffe en \hat{i} e mal ole ances an \hat{i} ei e \bullet essions of iffe en in s of \hat{i} is necessation of sea \hat{c}^{i} for mo e iffe en ial soos using mo e \hat{i} e moole an an susce \hat{i} de sil uo m o est. A i ional me \hat{i} o s fo \hat{i} e \hat{o}^{i} e sil uo m issues s'oul also de e do e . n \hat{i} e fut e \hat{i} e efo e use uill in estiga e \hat{i} e effects of e cessi e \hat{i} ea \hat{s}^{i} oc on \hat{i} e \bullet o eome of iffe en o est an se es.

Fu¹ e mo e ¹ e e a e man, successful e e imen s on ansgenic sil voom. To ve e 'i is onl, ecen l, ¹ a scien is s a e ec¹ nicall, cava'de of a geing en ogenous genes wien enginee ing 'ansgenic sil voom 14. T⁴ e efo e ma nivulaion of genes ela e o o'ds ness an ¹ e mo ole ance of sil voom is no oo fa avay. An un e s an ing of ¹ e molecula mec¹ anisms of ¹ e mal ole ance is essen ial fo a aning an, esul s in ¹ is i ec ion va icula l, in ¹ e un e s an ing ¹ e iffe en ial e ession va e n of 'a ious ⁵ S' s in 'j ol ine an mul i ol ine 'o ee s. T¹ e im vo ance of ¹ S'⁷ 0 v¹ ic¹ vas confi me fo sil voom la ae ¹ e mo ole ance in ¹ e \bullet esen esea c¹ woul g ea ly facili a e ¹ is esea c¹.

Acknowledgements We \hat{a} an ian ing i $\hat{a} \text{ ong}^{2}$ ia \hat{a}_{04} an Fang i fo \hat{a} ei ec nical \hat{a} el \hat{a} in e e imen s. Fi nancial si to \hat{b}_{0y} \hat{e} Deva men of Science an Tec nology \hat{a} biang \hat{n} o ince \hat{C} ina $(\hat{c} \cap C \cap \hat{c} \otimes \hat{b})$ an a ional asic Desea \hat{c} \hat{n} og am of \hat{C} ina $(\hat{c} \cap C \cap \hat{c} \otimes \hat{b})$ is g a efully ac nowle ge.

Pecei e a $c^{T} 25 2.00$ acceve $ul_{v} 2.00$

REFERENCES

- 1 aga h (2 0 (2) A volica ion of gene ic incides fo im• o ing sil • o µc ion. Curr. Sci. ? 40-414
- Fe ei a A. S. . P. T ola . C. . as_h, a . F. A a) o an A. C. o ges (205) Small¹ ea s'oc o eins in ¹ e e elo•men of ¹ e mo ole ance in *Pisolithus* s• J. herm. Biol. ° (5.5 ° Q.
 Pa sell D. A. an S. in his (1...?) T¹ e function of
- Pa sell D. A. an S. in tis (1...?) T⁴ e function of ¹ ea s³ oc • o eins in s ess ole ance. eg a a ion an eac i a ion of amage • o eins. Annu. e. enet. 27: 477 4...
- 4 e's P. A. an . . Fe e $(1 \sim)$ To Can la al ^a e mo ole ance in *Droso hila melanogaster*.^a owm_b \hat{c}^{t} is enoug \hat{g} an \hat{d} en is mo e oo m_b \hat{c}^{t} *J. nsect Ph siol.* 44 10 1 1101
- 5. evaman A. . . . Foe se . . S^T oema e an
 ■. . ■o'e son (2007) S ess in tree ^T e mo ole ance of en ila o , mo o va e n gene a ion in ^T e locus ocusta migratoria. J. nsect Ph siol. 4. 107 1047.
- . Fe e . . an . . "of mann (1....)" ta s' oc o eins molecula c'are ons an ¹ e s ess essonse. e o lu iona an ecological d'ysiology *Annu. e . Ph siol.* 12422.
- 7. S^{i} ilo a . . D. . a_{out}^{i} . . gen e an O. . a sevina (200) Small² ea sⁱ oc • o eins an a a•a ion of a ious *Droso hila* secies o² ye² e mia. *ol. Biol.* $402^{5}2^{2}$.
- . Stin W. . an on ag_{ij} an . e'_{0} iggen (202) Small² ea s² oc \bullet o eins an s ess ole ance in \bullet an s. *Biochim. Bio h s.* Acta 577. 1.
- e's \blacksquare . A. an \square \blacksquare e encou (1....) oly ion of \square e mo ole ance an a ia ion in \square e \square ea s¹ oc \blacksquare o ein \blacksquare **B**¹7 **C A**m. ool. \square \square \square **C I**.
- 10, Wang . "I an . ang (2005) Differences in egg ¹ e mo ole ance 'e ween ovical an emwe a e vovula ions of ¹ e mig a o , locus ocusta migratoriam (O ¹ ove a. Ac i ii ae)." *I. nsect Ph siol.* 5 1, 277 2 5.
- 11 Pobe son P. . (2 004) T^2 e mals ess an neu al function. a avi e med anisms in insec mo el systems. J. herm. Biol. 2: 25125.
- 2. e en . . (2 000 ¹⁷, siological es conses of insec s o¹ ea . Posthar est Biol.' echnol. 2 i 10 111.

- Γ. Fe e . . an ■. A. e's (1 · ·) a µ al an gene ic enginee ing of ¹ e¹ ea s¹ oc • o ein ¹ s of Cin Droso hila melanogaster. Conse µences fo ¹ e mo ole ance. Am. ool. ² . ⁵ C ⁵ Π
- 14 Dai T D. iang . Wang . L. Cao . Wang an . Fei (2007) De elowmen of a^{-1} ea s^{-1} oc in Lci'Je an im e i a'de D Ai s's em in sil vo m. *Biomol. ng.* 24 (25 \leq 0
- 5. σ^{T} mann C. F. an . Pi ifo (1.2) T^{T} e a s^{T} oc estonse an T ea sensi i i y of Bom mori. Sericologia 2.57 577.
- 1. $o_y \oplus an$. n owina⁷ an (1 > 5) Tea s⁷ oc e swonse in multiply sil wo m aces wi⁷ iffe en ⁷ e mo ole ances. *I. Biosci.* 2 (4 > 5 n).
- I7. C^f a a i . A. T Sosalegow a an . T o egow a (200) more of a s^f oc on f ea s^f oc
 o eins e ession 'jological an comme cial ai s of Bom mori. nsect Sci. P. 24 25 Q.
- 1. i . . . ia "Frii . anno an C. (205) • ession of "e small" ea stoc • o ein m⁴ s• 1... gene in sil vo m (Bom mori). Chin. J. Agr. Biotechnol. P. 1.5 201
- Song . T.S. . ung . ■. Seo S. W. ang an S. S. Tan (200) en ification of une egula e no eins in ¹ e¹ emo 1, mJ of immunite Bom mori la ae. Com . Biochem. Ph siol. Part D enomics Proteomics 12 (2).
- **2 C** a es \mathcal{P} . **D**. an T. A. . **T**₄ sea (**2 C0**) olecula biologis s gui e o \bullet o eomics. *icro iol. ol. Biol.* $e \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot$ **2** 1. a fo . . (1.7⁻) A a **u** an sensi i e me³ o
- 21. a fo . . (1.7⁻) A a vi an sensi i e me⁴ o fo ⁴ e tan i a ion of mic og am tali ies of • o eins tillking ⁴ e • incide of • o ein type tin ing. *Anal. Biochem.* 72:24 254
- 2². Uma e i . an **D**. **P**. **P**ao (2005) alga ion an se

lec ion of multi ol ine 'o ee ing esou ce ma e ials of sil vom *Bom mori* fo 'ig' em e au e an 'umi i con i ions. *I*. *ool*. : ?? ?? . ? 4 uma '. S. 'I. asa a a . alvana .

- 25. a o . . aga ast W. Ta a an O. inagi (1 < .)ffec of e vost e of e sil wo m Bom mori o igi enve at e on st i al a e an cocoon c a ac e. I n. Agr. es. $(2.5)^{-1}$
- *n. Argr. es.* . ??. < 1 < 4
 ?. Tsie⁷ F. S. S. S. S. an S. . reng (1.-5) S. ies on ⁷ e⁻⁷ e mo ole ance of ⁷ e sil vo m *Bom mori. Chin. J. ntomol.* 5. > 1 101
- 27. S Jas D. . S. . Co well n. . "Thins a sen an D. . asseal (2 (2)) "Tea s' oc of w ea u ing g ain filling. o eins associa e wi² a ea ole ance. J. Cereal Sci. °5. 175 1 .
- 2. o_{in} in a $n \ge n$. i_{ma} matrix a same $n \ge 1$. Sint a an T^{4} anga el₄ (2.00) Sc eening of \bullet omising ge malasm of $\bullet o_{i}$ ol ine sil wo m (Bom mori .) fo T^{4} e mo ole ance. n ian I. Sericulture 2 = 7.70
- 2... S le A. F. an o'ae s . The s . an eetimen an . De eese (2 (1)4 h o eomic analysis of small ² ea s² oc o ein isofo ms in 'a leg s² oo's. *Ph tochemistr* 5: 15° 1 °?.
- ° C Sa ano D. . i . ia . amamo o ™ Fµii an . Aso (2 CC) enes enco ing small ² ea s² oc • o eins of ² e sil uo m *Bom mori. Biosci. Biotechnol. Biochem.* 7 C 2 44 2 5 C