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Studies on the Thermotolerane of the Silkworm, Bombyx mori【Research report】

家蠶(Bombyx mori)耐熱性之研究【研究報告】

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Abstract

Larvae of two polyvoltine and four bivoltine silkworm strains were reared at the room temperature (25±1℃), high temperature (36±1℃), and flucturated temperature at 12hr intervals, respectively. The results showed that, besides the lst instar, the high temperrature treatment affected the survival rate more seriously than the others. The polyvoltine strains were more tolerant to heat than bivoltine strains at the 4th and 5th instars, and pupal stage. Among bivoltine strains, the Chinese race 〝Feng〞 was the most tolerant strain, followed by the Japanese races 〝Kuo〞 and 〝J-09〞 ; and the Chinese race 〝C-54〞 was the most susceptible one. Th cocoon shell weight percentages were found to be not significant different among treatments in polyvoltine strains, but were different in bivoltine strains. A reduction in silk yields was found in the treatments of the high and fluctuated temperatures. Newly-hatched larvae, after heated in water bath, were placed inside of glass tubes and maintained at 44, 45, 45.5, and 46℃ for 1hr, respectively. The mortality were 0, 12, 87, and 100%, respectively. Mortality caused by severe heat-shock treatments could be reduced by a prior exposure of larvae to a sublethal heat-shock treatment at 40℃ for 30min-8hr. After heat shock treatment of 40℃ for 6-8 hours, followed by a 25℃ recovery period (2hr) and a subsequent 47℃/ 1hr shock treatment, all larvae were found to survive easily. Same results were shown in the different instars, at lower lethal temperatures. Fat-body cells and hemocytes of fifth-instar larvae were collected after a severe 40℃/1hr shock followed by a 25℃ recovery period (2hr), and their proteins were compared with O'Farrel's two-dimensional gel electrophoresis. Three heat-shock-specific spots were found, and their molecular weight were about 70kD. Although the normal physiological temperature of the fifth instars ranged from 22 to 25℃, they could produce the same heat-shock-specific spots as the temperature elevated to a 7℃ higher. The thermotolerant silkworm strain Nong appeared the heat-shock-specific spots after a 32℃/1hr shock, but the nontolerant strain C-54 didn't.

摘要

以二個多化性及四個二化性家蠶品系，在室溫(25±1℃)、高溫(36±1℃)及室溫與高溫每隔12小時更換一次之變溫中飼養，比較品系及齡期間耐熱性之差異及其與吐絲量之關係。結果，除一齡蠶外，其餘各齡期均以持續高溫降低存活率最大，變溫次之。品系間以二個多化性品系較耐熱；二化性之中國種Feng次之，其次為日本種Kuo 及J-09，而中國種之C-54最差。齡期別則以一齡蠶最耐熱，二齡次之，五齡蠶最差。吐絲量方面，多化性家蠶在三種溫度處理間無顯著差異，而二性家蠶則在高溫及變溫中飼養較在室溫飼養者顯著減少。將剛孵化之家蠶幼蟲分別置於44、45、45.5、及46℃之高溫中，1hr後調查其死亡率，結果分別為0、12、87、及100%。若在上述致死高溫處理前，先將幼蟲置於40℃中30min至8hr後移回室溫(25℃)2hr，再以致死高溫衝擊1hr，則發現致死高溫可提高至46℃以上。其中以40℃處理6至8hr者，在47℃衝擊1hr仍保持100%之存活率；其他齡期亦有相似結果，惟致死溫度較低。家蠶五齡幼蟲以40℃熱衝擊1hr，再置回室溫2hr，取其脂肪細胞與血球細胞在細胞分離液中研磨、萃取，並以雙向電泳分離，明顯看出均產生三種以上熱衝擊蛋白(heat shock proteins, HSPs)；其分子量在70kD附近。試驗顯示，五齡蠶生長適溫為22至25℃，若調高7℃均可使蠶體產生相同的熱衝擊蛋白。飼養於室溫25±1℃之五齡家蠶幼蟲，經32℃熱水浴衝擊處理1小時後再以雙電泳分析，明顯看出耐熱性高之品系(Nong)比耐熱性差之品系(C-54)產生較多量70kD群之熱衝擊蛋白。

Key words: Bombyx mori, thermotolerance, heat shock proteins.

關鍵詞: 家蠶、耐熱性、熱衝擊蛋白。

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家蠶(*Bombyx mori*)耐熱性之研究

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摘 要

以二個多化性及四個二化性家蠶品系，在室溫(25±1℃)、高溫(36±1℃)及室溫與高溫每隔12小時更換一次之變溫中飼養，比較品系及齡期間耐熱性之差異及其與吐絲量之關係。結果，除一齡蠶外，其餘各齡期均以持續高溫降低存活率最大，變溫次之。品系間以二個多化性品系較耐熱；二化性之中國種Feng次之，其次為日本種Kuo及J-09，而中國種之C-54最差。齡期別則以一齡蠶最耐熱，二齡次之，五齡蠶最差。吐絲量方面，多化性家蠶在三種溫度處理間無顯著差異，而二化性家蠶則在高溫及變溫中飼養較在室溫飼養者顯著減少。將剛孵化之家蠶幼蟲分別置於44、45、45.5、及46℃之高溫中，1hr後調查其死亡率，結果分別為0、12、87、及100%。若在上述致死高溫處理前，先將幼蟲置於40℃中30min至8hr後移回室溫(25℃)2hr，再以致死高溫衝擊1hr，則發現致死高溫可提高至46℃以上。其中以40℃處理6至8hr者，在47℃衝擊1hr仍保持100%之存活率；其他齡期亦有相似結果，惟致死溫度較低。家蠶五齡幼蟲以40℃熱衝擊1hr，再置回室溫2hr，取其脂肪細胞與血球細胞在細胞分離液中研磨、萃取，並以雙向電泳分離，明顯看出均產生三種以上熱衝擊蛋白(heat shock proteins, HSPs)；其分子量在70kD附近。試驗顯示，五齡蠶生長適溫為22至25℃，若調高7℃均可使蠶體產生相同的熱衝擊蛋白。飼養於室溫25±1℃之五齡家蠶幼蟲，經32℃熱水浴衝擊處理1小時後再以雙向電泳分析，明顯看出耐熱性高之品系(Nong)比耐熱性差之品系(C-54)產生較多量70kD群之熱衝擊蛋白。

關鍵詞：家蠶、耐熱性、熱衝擊蛋白

前 言

家蠶一般在7-40℃間可以存活，而實用養蠶溫度範圍則在20-30℃左右(Ueda, 1976)。因本省地處亞熱帶及熱帶，晚春至秋季溫度常超過30℃以上，故此期間桑樹雖生長快速，產葉量最多，蠶作卻較不安定。又目前世界養蠶重心已逐漸南移至東南亞、印度等熱帶地區，該地區原飼養較耐熱之多化性家蠶品種，近年爲提高蠶繭產量及品質，

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亦多引進二化性品種進行改良或購買台灣、日本、韓國等地區之蠶卵。因此，家蠶耐熱性之研究亟需加強。據報告，家蠶品種於25或32℃中飼養時，其健蛹率並無顯著差異，但36℃高溫飼養時，則生長發育甚差，且品種間有明顯的差異(Ninaki *et al.*, 1987)。因某些生物在高於正常溫度5℃ (Mizzen and Welch, 1988; Raschke *et al.*, 1988)或10℃ (Chen, 1991)的環境下，原來在正常溫度時活躍的基因會受到抑制，而另一組新合成稱爲熱衝擊蛋白(heat shock proteins, HSPs)的基因則被活化，以保護重要的胞器，使其能耐較高溫。Ooi (1969)則指出，在高溫中生存率較高之家蠶品系，其產絲量有較低之趨勢。因此，筆者等假設，家蠶耐熱性品系在高溫逆境下，可能因產生HSPs以增強其耐熱能力，但亦致其正常合成絲蛋白的量減少。惟究竟家蠶在何種溫度下會產生HSPs，產生以後對家蠶耐熱能力之提高程度，及不同品系之間有無差異等均值得探討，以供養蠶技術改進及品種改良之參考。

材料與方法

一、不同品系及齡期家蠶之耐熱性比較

供試家蠶品系採用多化性之Yeiby、Jinhar，二化性中國系統之Feng、C-54，日本系統之Kuo、J-09等6個品系。將各品系之蠶同時飼養在25℃之蠶室中，各齡眠起脫皮後，逢機取100隻，移至36℃及36℃與25℃每隔12hr變換一次之環境中飼養，並以繼續留在25℃中者爲對照。每處理重複3次，調查及比較不同品系、齡期在各種溫度處理之存活率、五齡蠶處理後之結繭、化蛹及羽化後產卵情形。

二、家蠶熱衝擊蛋白(HSPs)之探討

(一)家蠶不同齡期幼蟲之致死高溫調查

以25℃飼養一至五齡之四元雜交品種「國・富×農・豐」之幼蟲，於每齡蟲初期，逢機取10隻，放入盛有桑葉之試管內，用保鮮膜封住管口，分別置於41-46℃(每間隔1℃爲一變級)之水浴中，1小時後取出，放回25℃養蠶室。隔夜(約經15hrs)，俟昏迷但未死之蠶已恢復活動後，調查其存活率，並以裝入試管後，繼續留置25℃者爲對照，每處理重複6次。

(二)家蠶幼蟲經非致死高溫處理與否之耐熱性比較

1.二化性四元雜種國・富×農・豐剛孵化之幼蟲(蟻蠶)先以38-42℃之非致死高溫衝擊30-240分鐘後，移回25℃中2小時，再以47℃之正常致死高溫衝擊1小時，調查比較其存活率。

2.以二化性中國系統Nong及日本系統Kuo之五齡第三日及老熟(約滿七日)幼蟲，先置於39℃之非致死高溫中衝擊1小時後，移回25℃中2小時，再置入44℃之正常五齡致死高溫中1小時，調查及比較經非致死高溫處理後於致死高溫中之存活率，及不同品系、不同日齡蠶之耐熱性差異。

(三)家蠶不同品系、飼養溫度、組織器官產生HSPs之分析比較

1.將25℃飼養之四元雜交品種「國・富×農・豐」之五齡家蠶幼蟲放入試管中，以40℃水浴熱衝擊1小時，再置回室溫2小時後，取其頭部、血球及脂肪體；將每公克組織細胞加入5ml細胞溶解液(配法如下)研磨萃取，參考O'Farrel(1975)方法，進行雙向電泳(two-dimensionsal gel electrophoresis)分析。先以樣本萃取液15ul進行IEF電泳，再將SDS電泳膠體以硝酸銀染色，分析比較蛋白質圖譜。

細胞溶解液配法

Urea	2.85g
NP-40	1ml
2-mercaptoethanol	250ul
Ampholine 3-10	250ul
H_2O	3.5ml

2.飼養於22℃及25℃之家蠶幼蟲，生長至五齡時分別以28-43℃之溫度熱衝擊，再取其脂肪體進行雙向電泳分析其蛋白質圖譜，探討溫度變化與家蠶HSPs生成之關係。

3.將家蠶品系C-54(不耐熱品系)及Nong(耐熱品系)，分別以40℃及32℃熱衝擊20及60min，再以雙向電泳分析其蛋白質圖譜，探討蠶品系耐熱性與HSPs生成之關係。

結　　果

一、不同品系及齡期家蠶之耐熱性比較

如表一所示，一齡蠶除二化性之中國系統C-54在36℃之高溫中存活率較差外，其餘五個品系與在25℃飼養之對照處理間並無顯著差異。二齡蠶在36℃高溫中飼養者，除日本系統之J-09及Kuo兩品系外，其餘四個品系則較對照處理者差。而一、二齡蠶在25及36℃交互更替中飼養者，除一齡之C-54及二齡之Jinhar兩品系較對照處理差外，其餘品系則與對照處理間無顯著差異。三、四、五齡蠶飼養在36℃高溫中之存活率，則除二個多化性品系之五齡蠶外，其餘均顯著低於

表一　高溫及變溫飼育對不同品系及不同齡期家蠶存活率之影響

Table 1. Effects of various rearing temperatures on survival rates of each instar of six silkworm varieties.

Temperature	Survival rate（%）1)2)					
（℃）	Jinhar	Yeiby	J-09	Kuo	Feng	C-54
1st instar						
25	96.67^{a}_{AB}	91.11^{a}_{B}	98.89^{a}_{A}	98.89^{ab}_{A}	94.44^{a}_{AB}	100.00^{a}_{A}
36	96.67^{a}_{A}	82.22^{a}_{A}	96.67^{a}_{A}	100.00^{a}_{A}	94.44^{a}_{A}	76.67^{b}_{B}
25～36	81.11^{a}_{B}	84.44^{a}_{B}	96.67^{a}_{A}	93.34^{b}_{AB}	96.67^{a}_{A}	90.00^{b}_{AB}
2nd instar						
25	90.00^{a}_{AB}	80.00^{a}_{B}	83.33^{a}_{AB}	92.22^{a}_{A}	94.44^{ab}_{A}	88.89^{a}_{AB}
36	68.89^{b}_{BC}	60.00^{b}_{C}	83.88^{a}_{A}	95.56^{a}_{A}	73.33^{b}_{B}	49.89^{b}_{C}
25～36	77.78^{b}_{B}	77.78^{a}_{B}	96.67^{a}_{A}	95.56^{a}_{A}	96.67^{a}_{A}	94.45^{a}_{A}
3rd instar						
25	91.11^{a}_{AB}	97.78^{a}_{A}	98.89^{a}_{A}	91.11^{a}_{AB}	87.78^{a}_{B}	97.78^{a}_{A}
36	37.78^{b}_{A}	17.78^{b}_{A}	17.78^{b}_{A}	26.67^{b}_{A}	32.32^{b}_{A}	0.00^{b}_{B}
25～36	91.11^{a}_{A}	86.67^{a}_{A}	88.89^{a}_{A}	91.11^{a}_{A}	87.78^{a}_{A}	88.89^{a}_{A}
4th instar						
25	89.50^{a}_{B}	97.00^{a}_{A}	98.25^{a}_{A}	97.50^{a}_{A}	96.25^{a}_{A}	87.78^{a}_{B}
36	12.25^{b}_{B}	67.00^{b}_{A}	0.00^{a}_{C}	19.00^{c}_{B}	0.00^{c}_{C}	0.00^{c}_{C}
25～36	90.00^{a}_{A}	92.22^{a}_{A}	54.75^{b}_{B}	83.00^{b}_{A}	85.50^{b}_{A}	43.00^{b}_{B}
5th instar						
25	80.50^{a}_{A}	91.50^{a}_{A}	80.25^{a}_{AB}	77.75^{a}_{B}	93.50^{a}_{B}	38.75^{a}_{C}
36	78.00^{a}_{B}	81.25^{a}_{A}	50.25^{b}_{D}	26.25^{b}_{D}	93.25^{b}_{C}	0.00^{b}_{E}
25～36	73.50^{a}_{B}	87.00^{a}_{A}	24.50^{b}_{D}	19.50^{b}_{D}	56.50^{b}_{C}	0.00^{b}_{E}

1) Means within a columm in the same instar followed by the same letters are not significantly different at 1% level according to Duncan's new multiple range test（DMRT）.

2) Means with the same capital letters in the same row are not significantly different at 1% level according to DMRT.

25℃之對照處理者。三齡之蠶在25℃與36℃之溫度間交替飼養者，各品系與對照區間均無顯著差異，而四齡蠶則除Jinhar及Yeiby外，其餘各品系之存活率都較完全在25℃中者差，但三、四齡蠶在變溫中飼養結果，均比始終在36℃高溫中者存活率高；五齡蠶則除二個多化性品系外，變溫處理均較25℃中者差，且與36℃高溫中者差異不顯者。

在吐絲量方面，由表二知，二個多化性品系之五齡蠶，在三種不同溫度中飼養結果，其單粒繭重及繭層率(吐絲量÷繭重×100)並無顯著差異；其餘四個二化性品系在36℃高溫中飼養者，只有中國系統之〝Feng〞有部份結繭，但吐絲量僅及在25℃中飼養者之三分之一強；另外三個品系則僅少量零散吐絲，未結成蠶繭。而在25與36℃交替環境中飼養之四個二化性品系之吐絲量則比在25℃中飼養者差，惟比始終在36℃中飼養者表現良好。

二、家蠶熱衝擊蛋白之探討

(一)家蠶不同齡期幼蟲之致死高溫比較

將飼養於25℃中之家蠶現行推廣品種「國·富×農·豐」之幼蟲，於每齡眠起脫皮後，逢機取出，置於41-46℃，每隔1℃爲一變級之高溫中1小時後，除41及42℃兩處理外，在43-46℃中之各齡期幼蟲多呈昏迷狀態。經移回25℃中隔夜，使存活之幼蟲恢復活動，調查其存活率，結果如表三所示。五齡幼蟲在43℃高溫中1小時，即有部份個體死亡，在44℃以上高溫中1小時則全部死亡。三、四齡幼蟲在43℃中1小時仍全部存活，44℃以上死亡率漸增，至46℃則全部死亡。一、二齡幼蟲則在44℃以下高溫中1小時仍能全部存活，至45℃才有部份死亡，而在46℃中1小時亦全部死亡。

(二)家蠶幼蟲經非致死高溫處理與否之耐熱性比較

由表四數據知，剛孵化之家蠶幼蟲以38℃前處理30-240分鐘後再置於47℃者，全部死亡；以42℃處理者，四種處理均有部份個體存活，惟存活率均未超過50%；而以40℃衝擊120及240分鐘之處理，則置於47℃

表二 五齡及營繭期不同溫度對蠶繭單粒重、繭層率及健蛹率之影響

Table 2. Effects of various temperatures on the single cocoon weight, percentage of cocoon shell weight and survival rates of pupae during the 5th instar and cocooning period.

Temperature(℃)	Jinhar	Yeiby	J-09	Kuo	Feng	C-54
			Cocoon weight (g)			
25	0.72	0.65	1.38	1.49	1.25	1.30
36	0.66	0.62	*1)	*	1.17	*
25~36	0.69	0.65	1.25	0.86	1.04	*
			Cocoon shell weight (%)			
25	11.90	13.39	20.36	21.22	20.72	20.91
36	11.14	14.66	*	*	7.77	*
25~36	12.83	13.32	11.26	13.86	18.73	*
			Survival rates of pupae (%) 2)			
25	73.50a	77.50a	65.50a	59.50a	55.25a	13.13a
36	21.76c	35.50b	0.00b	0.00c	0.00c	0.00b
25~36	53.50b	78.50a	0.00b	2.75b	12.75b	0.00b

1) No cocoon was completed.
2) Means with the same letter of the same column are not significantly different at 1% level according to DMRT.

表三　各齡期家蠶幼蟲以不同高溫衝擊一小時後之存活率

Table 3. The survival rate of larvae of *Bombyx mori* at various heat shock for 1hr

Temperature (℃)	Survival rate (%)[1) 1st instar	2nd	3th	4th	5th
43	100.00±0.00	100.00±0.00	100.00±0.00	100.00±0.00	63.33±24.21
44	100.00±0.00	100.00±0.00	81.67±9.83	41.67±9.83	0.00±0.00
45	88.33±11.67	71.67±15.60	40.00±8.94	21.67±14.24	0.00±0.00
46	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00

1) Survival rates were investigated at the 15th hour after heat shock.

表四　以數種非致死高溫前處理後對蟻蠶在47℃之正常致死高溫中存活率之影響

Table 4. Effect of preincubation at non-lethal high temperatures on the survival rate of *Bombyx mori* neonates at 47℃

Preincubation temperature (℃)	Survival rate (%) Duration of preincubation (min) 30	60	120	240
38	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
40	0.00±0.00	0.00±0.00	83.33±15.05	95.00±8.36
42	40.00±16.73	13.33±8.16	31.66±11.69	45.00±13.78

Each preincubation treatment was followed by a 2hrs chase at 25℃ and then transferred to heat shock at 47℃ for 1hr. The survival rates were investigated at the 15th hour after heat shock. Sixty larvae were examined in each treatment.

表五　不同的40℃前處理時間對蟻蠶在正常致死高溫中存活率之影響

Table 5. Effect of various durations of preincubation at 40℃ following a 2hrs chase at 25℃ and then transferred to heat shock at 46, 46.5, or 47℃ for 1hr on the survival rate of *Bombyx mori* neonates.

Duration of 40℃ incubation(min)	Survival rate (%)[1) 46.0	46.5	47.0
0(ck)	0.00±0.00	0.00±0.00	0.00±0.00
30	100.00±0.00	100.00±0.00	0.00±0.00
60	100.00±0.00	100.00±0.00	0.00±0.00
120	100.00±0.00	100.00±0.00	83.33±15.05
240	——	——	95.00±8.36
360	——	——	96.67±8.16
480	——	——	100.00±0.00

1) The survival rates were investigated at the 15th hour after heat shock, and 60 larvae were examined in each treatment.

後仍有83％以上之存活率。因此，進一步以40℃之非致死高溫爲前處理，調查其能忍受正常致死高溫46-47℃所需處理時間。結果由表五知，在46.5℃以下之正常致死高溫中1小時內，只要先以40℃前處理30分鐘以上，即可獲得100％之存活率；但若溫度升高到47℃，則需要120分鐘之前處理，才能使其後在47℃中衝擊1小時之存活率達到83.33％。

爲了解相同齡期中不同日齡幼蟲之耐熱能力是否亦有差異，乃以二化性中國系統

表六　以39℃之非致死高溫前處理後，對不同家蠶品系五齡中期及末期幼蟲在44℃之正常致死高溫中存活率之影響

Table 6. Effect of preincubation at 39℃ for 1hr following a 2hrs chase at 25℃ and then transferred to heat shock at 44℃ for 1hr on the survival rate of different *Bombyx mori* strains at 5th instar.

Strain Treatment[1]	Survival rate（%）	
	5th instar 3rd days	matured larvae
Nong t	66.67±5.16	90.00±12.64
ck	0.00±0.00	16.67± 8.68
Kuo t	0.00±0.00	56.67±40.28
ck	0.00±0.00	6.67± 6.14

1) t=rearing at 25℃→39℃ for 1hr→25℃ for 2hr→44℃ for 1hr→25℃ for 15hr
ck=rearing at 25℃→44℃ for 1hr→25℃ for 15hr. Sixty larvae were examined in each treatment.
→：moved to

表七　不同熱衝擊處理對家蠶脂肪體產生熱衝擊蛋白之影響

Table 7. Heat-shock proteins synthesis in *Bombyx mori* fat body in different heat-shock treatments.[1]

Rearing temperature (℃)	Treatment temperature (℃)	HSPs synthesis
25	43	+
	40	+
	35	+
	32	+
	28	−
	25	−
22	37	+
	29	+
	28	±
	22	−

1) Fifth-instar of bivoltine silkworm were exposed to a 1hr heat-shock treatment followed by a 2hr recovery at rearing temperature.

表八　不同家蠶組織之熱衝擊蛋白產生情形

Table 8. Heat-shock proteins synthesis in *Bombyx mori* tissues[1]

Tissue	HSPs synthesis
Fat body	+++
Hemocytes	+
Head tissues	+

1) Fifth-instar were treated with 40℃/1hr shock followed by a 2hr recovery at 25℃.

Nong及日本系統Kuo等二品系之五齡第三日及老熟幼蟲，分別先以39℃之非致死高溫處理1小時，及移回25℃靜置2小時後，再置於五齡初期之致死高溫44℃中1小時，以調查比較其存活情形。結果如表六所示，五齡中期之耐熱性較五齡末期之老熟幼蟲差，又相同齡期時日本系統Kuo之耐熱性較中國系統Nong差。

㈢家蠶不同品系、飼養溫度及組織器官產生HSPs之分析比較

為明瞭家蠶是否眞能產生熱衝擊蛋白及其產生條件，乃再度利用雙向電泳分析法，調查五齡家蠶幼蟲不同品系及不同飼養溫度變化，其蛋白質圖譜之變異情形。結果由表七知，以25℃飼養之五齡蠶，在25及28℃之溫度衝擊中，其蛋白質圖譜並無差異；但以32-43℃衝擊1小時後，則可在70kD附近看到比留在25℃中之對照組多出三個蛋白質點(圖五、六)；而飼養在22℃之蠶，則僅需29℃以上高溫衝擊1小時，即可產生類似效果。

又本實驗以五齡家齡家蠶經熱衝擊後，取其頭部、脂肪體及血液中之細胞分析，結果均能測得上述三種蛋白質；其中以從脂肪體中所獲得之蛋白質圖譜最為清晰(表八)。

當飼養於室溫25±1℃之中國系統較耐熱

表九　不同熱衝擊處理對不同家蠶品系熱衝擊蛋白產生之影響

Table 9. Heat-shock proteins synthesis in different *Bombyx mori* Strains in different heat-shock treatment.

Variety	Treatment Temperatme(℃)	Duration of incubation(min)	HSPs Synthesis
Nang	40	60	+
	32	60	+
	32	20	−
C-54	40	60	+
	32	60	−
	32	20	−

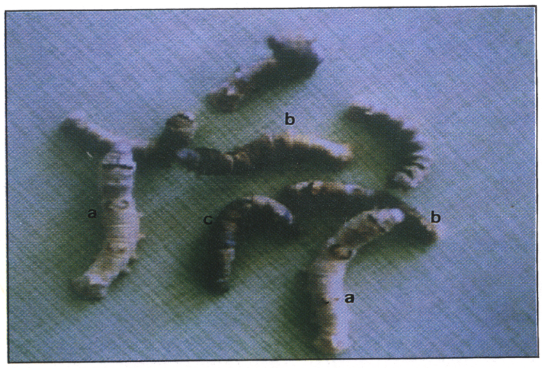
1) Fifth-instar were exposed to a heat-shock treatment followed by a 2hr recovery at rearing temperature.

品系Nong及較不耐熱品系C-54之五齡幼蟲，經40℃非致死高溫衝擊60分鐘後，分析其脂肪體中之蛋白質圖譜，結果二者均能產生前述70kD群之HSPs；但若以較低之32℃非致死高溫衝擊60分鐘，則僅較耐熱之Nong品系產生HSPs三點中之二點，C-54則全無發現；若僅以32℃衝擊20分鐘，則Nong及C-54都未有HSPs產生(表九)。

討　　論

根據試驗期間觀察，各齡期高溫造成之斃死蠶都集中在就眠前後，尤以眠中最多，且多爲不能脫皮或半脫皮致死。根據Riddiford and Truman(1978)報告，昆蟲眠起脫皮之際，由脫皮腺分泌之脫皮液注入新舊表皮內層，因其所含消化酶作用於舊表皮內層，使得新舊表皮能順利分離。本實驗所見之脫皮困難幼蟲，其外觀均呈黑褐色，與正常脫皮者迥然不同(圖一)，推測可能係高溫導致脫皮液中之消化酶或其他成分在合成過程遭遇阻礙或因高溫以致變性。

在36℃高溫下，終齡(五齡)幼蟲之死亡尖峰期與前四齡者不同，此期有兩個高峰。前者出現在五齡第5-6日，蠶兒減少取食、出



圖一　36℃高溫飼養下，四齡幼蟲眠後脫皮情形。a.正常脫皮b.半脫皮c.不能脫皮

Fig. 1. The exuviation of 4th instar after molt when reared at 36℃. a. Normal exuviate b. Semi-exuviate c. No exuviate

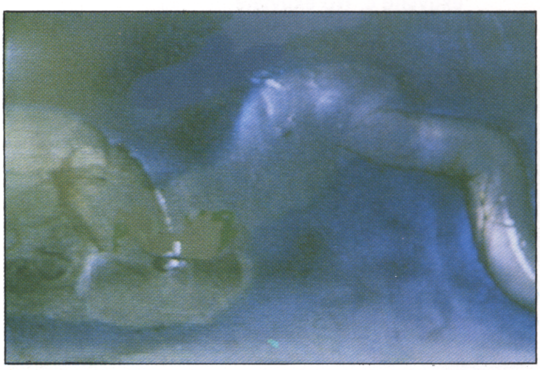
圖二　36℃高溫飼養下，五齡幼蟲下痢及體軀後部萎縮情形。

Fig. 2. Discharged soft feces and shrink in posterior body of fifth instar when reared at 36℃.

現下痢徵狀，體軀萎縮死亡(圖二)。第二高峰期出現在化蛹前，死蠶都屬不吐絲或僅散亂吐絲不能正常結繭者(圖三)；經解剖，可見其中部絹絲腺局部縮縊畸形(圖四)，可能因此導致吐絲過程中絲液輸送受阻。根據Ono (1940)報告，殘留體內的大部分絹絲於化蛹前二、三日若不能分解，將阻礙其化蛹或羽化而致死。Oshiki (1973)以38℃高溫衝擊四齡蠶2小時後，亦出現五齡老熟幼蟲之中部絲腺變異或後部絲腺退化導致不能結繭；

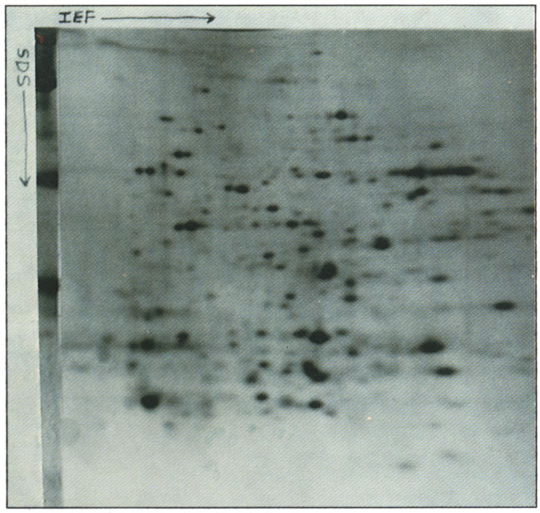
圖三　36℃高溫飼養後，五齡老熟幼蟲僅散亂吐絲即死亡。

Fig. 3. The dead 5th instar after spinning silk irregulately when reared at 36℃.



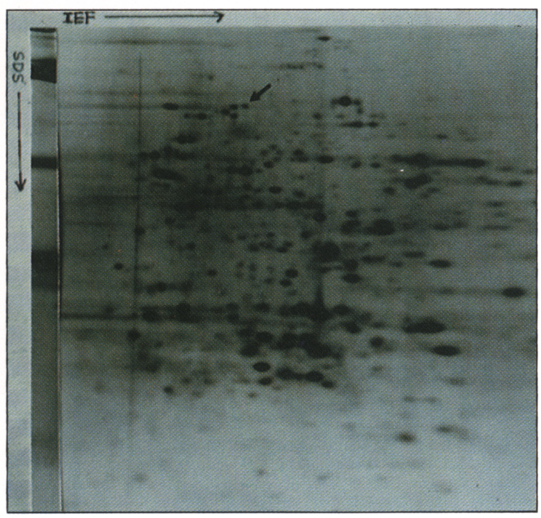
圖四　36℃高溫飼養後，不能吐絲之老熟幼蟲中部絲腺縮縊畸形。

Fig. 4. Narrowed in partial of the middle silk gland and failed in spinning of 5th mature instar when reared at 36℃.



圖五　室溫(25℃)下家蠶脂肪細胞內蛋白質之雙向電泳分析圖譜。

Fig. 5. Two-dimensional gel analysis of proteins synthesized in normal fat-body cells of *Bombyx* silkworm reared at 25℃.



圖六　高溫(40℃，1hr)熱衝擊下，家蠶脂肪細胞內蛋白質之雙向電泳分析圖譜。箭頭所指為熱衝擊蛋白。

Fig. 6. Two-dimensional gel analysis of heat-shock proteins synthesized in heat-shock treated (40℃ 1hr) fat-body cells of *Bombyx* silkworm reared at 25℃. The arrow indicates the heat-shock proteins.

經咽側體(corpus allatum)切除及移植試驗結果，認爲係因高溫導致青春激素(juvenile hormone, JH)分泌減少所致。因此推論本實驗飼養於高溫中不能吐絲結繭之五齡蠶，可能亦與咽側體之內分泌異常有關。

由試驗結果知，家蠶幼蟲五齡期間之耐熱性不僅不同品系間差異甚大，且較耐熱之品系內，其個體間亦有差異。因本試驗均在環境相當一致之蠶室內進行，因此推測品系間或品系內個體間之差異應以遺傳變異之成分居多。若果眞如此，則吾人可望從高溫飼養的五齡蠶中，篩選較耐高溫之品系，供高溫地區養蠶用。惟因健蛹率方面，如表二所示，五齡開始至化蛹期間，均置於36℃高溫者，除原產於熱帶之二個多化性品系有少部份化蛹及存活外，其餘二化性品系則均未獲得健蛹。且此二個多化性品系在36℃高溫中即使能結繭化蛹及羽化，交配產卵後亦全部產下不受精卵。根據Sugai and Takahashi(1981)報告，自開始吐絲至前蛹期，高溫最易引起雄不妊現象，蛹期次之。Chen and Lin (1985)指出，花粉在高溫下不能產生熱衝擊蛋白，因此雄性配偶子未能受到適當保護，致植物在高溫下不易完成受精作用。由本試驗結果知，家蠶在高溫下之不妊現象可能亦與此有關。因此，五齡蠶經高溫篩選後，自老熟幼蟲吐絲結繭開始至羽化交配產卵，都應避免高溫，俾便留種。

由試驗結果看出，家蠶幼蟲之高溫忍受性隨齡期增加而遞減。此結果與Ueda (1976)所指出養蠶適溫於一齡之幼蟲期較高，約27-29℃，隨齡期增加，每齡約遞減1℃，及Kuribayashi (1991)認爲，30℃以上的高溫，會擾亂蠶的正常生理機能，其中尤以對五齡蠶之影響最大之論點頗爲吻合。

一般生物體暴露在高於正常生長溫度某種程度以上之環境時，其原本正常活躍之基因即會受到抑制，另一組基因則被激活，產生所謂的熱衝擊蛋白，以保護細胞內各種胞器，並減少細胞內可溶性蛋白質在高溫中變性(Chen, 1991; Mizzen and Welch, 1988; Raschke *et al.*, 1988)。本報告前節曾述及剛孵化之家蠶幼蟲，以44℃之高溫衝擊1小時，有11.67%之個體死亡，而以46℃衝擊1小時，則全部死亡(表三)。本實驗先假設家蠶遇非致死高溫亦能產生HSPs，因此進一步以38-42℃之高溫衝擊剛孵化之幼蟲30-240分鐘後，移回25℃室溫2小時，令其產生HSPs，再移到47℃之正常必死高溫中衝擊1小時，調查其存活率增加情形，結果知家蠶幼蟲之耐熱性，與其在非致死高溫下之滯留時間有顯著關係。

Kosegawa *et al.*(1991)以46℃比重1.10之熱鹽酸浸漬經過5℃冷藏，胚胎已完全脫離休眠之蠶卵5分鐘，並以置於25℃未經浸酸之卵爲對照，結果高溫浸酸後亦產生與本實驗相似之三種熱衝擊蛋白，而對照處理則否。此一結果亦可佐證Chen and Lin (1985)認爲HSPs之產生不限於任何生長發育時期之說法應屬正確。

依試驗結果，不同家蠶品系間之耐熱性差異與其HSPs之產生效率呈正相關。因此，吾人於家蠶耐熱性品系選育之初期，當可供比較之品系相當多時，亦可望先藉由HSPs之定性或定量分析，來判別家蠶不同品系間耐熱性之差異，俟選出少數較耐熱之品系後，再進行高溫飼養及個體選拔。如此將可節省育種初期所需之大量養蠶空間、勞力及經費，並避免當空間及人力不足必需分批篩選時，因環境變異而增加試驗誤差。

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Studies on the Thermotolerance of the Silkworm, *Bombyx mori*

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ABSTRACT

Larvae of two polyvoltine and four bivoltine silkworm strains were reared at the room temperature (25±1℃), high temperature (36±1℃), and fluctuated temperature at 12hr intervals, respectively. The results showed that, besides the lst instar, the high temperature treatment affected the survival rate more seriously than the others. The polyvoltine strains were more tolerant to heat than bivoltine strains at the 4th and 5th instars, and pupal stage. Among bivoltine strains, the Chinese race "Feng" was the most tolerant strain, followed by the Japanese races "Kuo" and "J-09"; and the Chinese race "C-54" was the most susceptible one. Th cocoon shell weight percentages were found to be not significant different among treatments in polyvoltine strains, but were different in bivoltine strains. A reduction in silk yields was found in the treatments of the high and fluctuated temperatures. Newly-hatched larvae, after heated in water bath, were placed inside of glass tubes and maintained at 44, 45, 45.5, and 46℃ for 1hr, respectively. The mortality were 0, 12, 87, and 100%, respectively. Mortality caused by severe heat-shock treatments could be reduced by a prior exposure of larvae to a sublethal heat-shock treatment at 40℃ for 30min-8hr. After heat shock treatment of 40℃ for 6-8 hours, followed by a 25℃ recovery period (2hr) and a subsequent 47℃ / 1hr shock treatment, all larvae were found to survive easily. Same results were shown in the different instars, at lower lethal temperatures. Fat-body cells and hemocytes of fifth-instar larvae were collected after a severe 40℃ / 1hr shock followed by a 25℃ recovery period (2hr), and their proteins were compared with O'Farrel's two-dimensional gel electrophoresis. Three heat-shock-specific spots were found, and their molecular weight were about 70kD. Although the normal physiological temperature of the fifth instars ranged from 22 to 25℃, they could produce the same heat-shock-specific spots as the temperature elevated to a 7℃ higher. The thermotolerant silkworm strain Nong appeared the heat-shock-specific spots after a 32℃ / 1hr shock, but the nontolerant strain C-54 didn't.

Key word: *Bombyx mori*, thermotolerance, heat shock proteins

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