

localities in Guangdong: Huaxian, Songhua, Zhaozuan, Dongwan, Nanhai, Sanshui, Xinhui, Zhongzan, Pengyu, Huiyan, Chaoan, Lianshan, Fenkai,

Xinyi, Meixian, and Wuhua. Experiments were repeated many times. The GM populations were classified according to varietal reaction.

Reactions of the differential varieties to GM differed among locations. Results indicated there are at least four GM biotypes in the province (see table). □

### Detection of enzyme polymorphism among populations of brown planthopper (BPH) biotypes

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We found horizontal starch gel electrophoresis useful for surveying enzyme polymorphism among BPH biotypes.

The procedure has three steps.

- 1. Preparation of enzyme crude extracts.** Newly emerged male and female BPH biotype 1, 2, and 3 were obtained from stock cultures and frozen at  $-20^{\circ}\text{C}$  for at least 2 h before electrophoresis. Individual hoppers were placed in depressions on a spot plate and ground in 15  $\mu\text{l}$  of the homogenizing solution (0.05M tris-histidine buffer, pH 8) using a glass rod. Whatman filter paper no. 3 bits (4 mm  $\times$  9 mm) were used to adsorb the crude extract and were inserted directly into the appropriate gel.
- 2. Electrophoresis.** Standard horizontal starch gel electrophoresis was used. The starch gel (14%, Electro-starch) was prepared using 0.05M tris-histidine buffer pH 8 and was used to resolve all the enzymes except esterase, which separated more clearly at pH 6. Tris-citrate (0.4M, pH 8) was the electrode buffer, and was adjusted to pH 6 for esterase. Electrophoresis was conducted for 4 h in a refrigerator ( $0-4^{\circ}\text{C}$ ) at about 30 mA/gel slab. An ice pack was placed on top of the gel during electrophoresis; afterward, the gel was sliced horizontally into several sheets and stained.
- 3. Histochemical staining.** We used the following enzyme assays. *Alcohol*

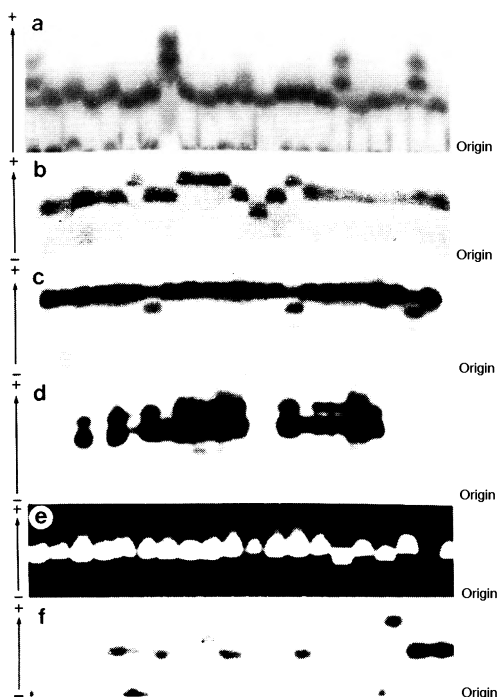
### Enzymes investigated after horizontal starch gel electrophoresis. IIRRI, 1983.

Locus	Enzyme activity <sup>a</sup>	Gene loci (no.)	Isoenzymes (maximum no.)	Migration
<i>Polymorphic</i>				
Catalase	+	1	2	Anodal
Esterase	+	3	6	Anodal
Isocitrate dehydrogenase	+	1	3	Anodal
Malate dehydrogenase	+	1	3	Anodal
Malic enzyme	+	1	4	Anodal
Phosphoglucose isomerase	+	2	8	Anodal
<i>Monomorphic</i>				
Acid phosphatase	+	1 or 2	2	Cathodal
Glucose kinase	+	1	1	Anodal
Glucosed-6-phosphate dehydrogenase	+	1 or 2	2	Anodal
Leucine aminopeptidase	+	1	1	Anodal
Phosphogluconate dehydrogenase	+	1 or 2	2	Anodal
Alcohol dehydrogenase	-			
Glutamate dehydrogenase	-			
Glutamate-oxaloacetate mutase	-			
Lactate dehydrogenase	-			
Peroxidase	-			
Shikimate dehydrogenase	-			
Tetrazolium oxidase	-			

<sup>a</sup> + = present, - = not detected.

*dehydrogenase (Adh)*: 1 mg PMS, 10 mg NBT, 10 mg NAD, 0.25 ml EtOH in 50 ml 0.05M tris-HCl buffer, pH 8.5, incubate gel for 30 min at  $24^{\circ}\text{C}$ . *Leucyl aminopeptidase (Lap)*: 25 mg leucyl- $\beta$ -naphthyl in 5 ml N,N'-dimethyl formamide, 25 mg FBK salt, 25 ml tris-maleate buffer (0.2M, pH 3.3), 20 ml NaOH (0.1 M), incubate gel for 30 min at  $40^{\circ}\text{C}$ . *Acid phosphatase (AcPh)*: 50 mg Fast garnet GBC, 50 mg  $\alpha$ -naphthyl acid phosphate, 0.5 ml  $\text{MgCl}_2$  (0.1M), 50 ml acetate buffer (0.2M, pH 4), incubate gel overnight at  $24^{\circ}\text{C}$ . *Catalase (Cat)*: 50 ml  $\text{H}_2\text{O}_2$  (7%) solution, incubate gel for 3 min, then wash with tap water, pour 50 ml KI solution (0.09M) with a drop of acetic acid. *Esterase (Est)*: 50 mg  $\alpha$ -naphthyl acetate, 10 mg Fast blue RR, 10 mg Fast garnet GBC in 50 ml phosphate buffer (0.1 M, pH 6.5), incubate gel for 15 min at  $40^{\circ}\text{C}$ . *Glucose-6-phosphate dehydrogenase (Gpd)*: 1 mg PMS,

10 mg NBT, 5 mg NADP, 2 ml  $\text{MgCl}_2$  (0.1M), 100 mg glucose-6-phosphate in 50 ml 0.05M tris-HCl buffer, pH 8.5, incubate gel for 30 min at  $24^{\circ}\text{C}$ . *Glutamate dehydrogenase (Gdh)*: 250 mg sodium glutamate, 10 mg NAD, 10 mg NBT, 1 mg PMS in 50 ml 0.05M tris-HCl buffer, pH 8.5, incubate gel for 30 min at  $24^{\circ}\text{C}$ . *Glutamate oxaloacetate transaminase (Got)*: 200 mg aspartic acid, 100 mg  $\alpha$ -ketoglutaric acid, 1 mg pyridoxal 5, phosphate, 200 mg Fast blue BB salt in 50 ml 0.05M tris-HCl buffer, pH 8.5, incubate gel for 30 min at  $24^{\circ}\text{C}$ . *Isocitrate dehydrogenase (Idh)*: 100 mg isocitrate tri-sodium, 5 mg NADP, 10 mg NBT, 1 mg PMS, 2 ml  $\text{MgCl}_2$  (0.1M) in 0.05M tris-HCl buffer, pH 8.5. *Lactate dehydrogenase (Ldh)*: 1 mg PMS, 10 mg NBT, 10 mg NAD, 1M/80 lillactate in 0.05M tris-HCl buffer, pH 8.5, incubate gel for 30 min at  $24^{\circ}\text{C}$ . *Malate dehydrogenase (Mdh)*: 1 mg



Zymograms of (a) malate dehydrogenase, (b) phosphoglucose isomerase, (c) isocitrate dehydrogenase, (d) esterase, (e) catalase, and (f) malic enzyme.

PMS, 10 mg NBT, 10 mg NAD, 10 ml malate (0.05M, pH 6) in 40 ml 0.05M tris-HCl buffer, pH 8.5, incubate gel in the dark for 15 min at 40°C. *Peroxidase (Pox)*: 15  $\mu$ l H<sub>2</sub>O<sub>2</sub> solution (30%), 1 ml CaCl<sub>2</sub> solution (0.1M), 2.5 ml N,N'-dimethyl formamide, 20 mg 3-amino-9-ethyl carbazole in 42 ml acetate buffer (0.05M, pH 5). *6-Phosphogluconate dehydrogenase (Pgd)*: 10 mg sodium phosphogluconate, 5 mg NADP, 10 mg NBT, 1 mg PMS, 2 ml MgCl<sub>2</sub> (0.1M) in 50 ml 0.05M tris-HCl buffer, pH 8.5, incubate gel in the dark for 15 min at 40°C. *Phosphoglucose isomerase (Pgi)*: 50 mg fructose-6-phosphate, 5 mg NADP, 10 mg NBT, 1 mg PMS, 2 ml MgCl<sub>2</sub> (0.1M), 3  $\mu$ l glucose-6-phosphate dehydrogenase in 20 ml tris-HCl buffer (0.5M, pH 8.5). Mix with 25 ml 2% agar

solution kept at 55°C and pour on the slice. Incubate in the dark for 15 min at 40°C. *Shikimate dehydrogenase (Sdh)*: 25 mg shikimic acid, 5 mg NADP, 10 mg NBT, 1 mg PMS in 50 ml 0.05M tris-HCl buffer, pH 8.5, incubate gel in the dark for 15 min at 40°C. *Superoxide dismutase (Sod)*: 15 mg NBT, 3 mg riboflavin, 4 mg EDTA, in 0.05M tris-HCl buffer, pH 8.5, incubate at 37°C in dark for 30 min, then expose to UV light. *Tetrazolium oxidase (To)*: 25 mg NAD, 20 mg NBT, 5 mg PMS, in 50 ml 0.05M tris-HCl, pH 8.5. Expose gel to light until white bands appear on blue background.

Using this technique, we investigated a total of 18 enzymes (see table). The figure shows the zymogram pattern of the 6 of 11 enzymes for which activity was noted. □

### Cytogenetics of the whitebacked planthopper (WBPH) *Sogatella furcifera* (Horváth)

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Cellular and karyotypic attributes such as chromosome number, morphology, morphometrics, and behavior involving position of chromosomes at various phases of cell division, differential contraction, bivalent configuration, etc., are important in understanding insect species relationships. We investigated the cytogenetics of WBPH. Oocytes and spermatocytes of insectary-reared, newly emerged adults were examined using the standard squash technique and lacto-aceto-orcein staining method.

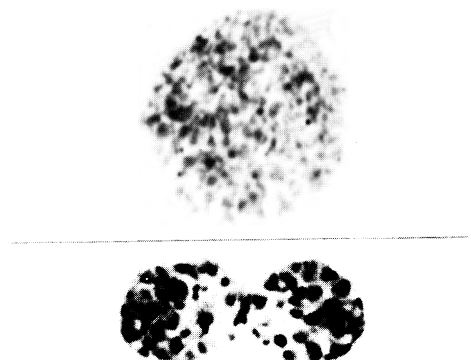
Reproductive cells simultaneously underwent both the conventional meiotic sequence and reverse meiosis, which led to the occurrence of uninucleated and binucleated meiocytes (Fig. 1). Binucleated cells resulted from failures of the tetrads of meiotic inversions to undergo complete cytokinesis. The frequency of

occurrence of binucleated cells in 15 males that were examined was 21 per individual, or about 24% of the average total (88) meiocytes detected. Uninucleated cells averaged about 50 per insect or almost 56% of the total dividing cells.

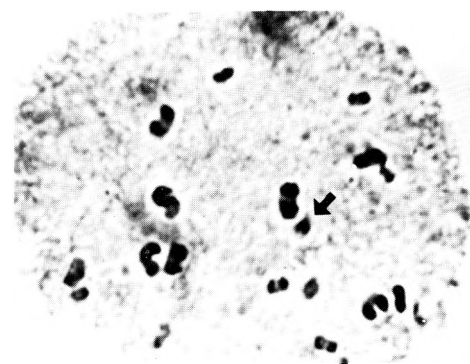
The uninucleated interphase cells were 47 $\mu$  long and 30 $\mu$  wide; the binucleated cells were 36 $\mu$  long and 19 $\mu$  wide. The nuclei of uninucleated cells were 5-10 $\mu$  long and 8-9 $\mu$  wide; the circular nuclei of binucleated cells had a 5 $\mu$  diameter. Of the two nuclei in binucleated cells, only one survived and proceeded to the reductional division of meiosis. The other nucleus degenerated rapidly and was lost before metaphase I.

The mean number of dividing and nondividing testicular cells observed per individual was 88 and 94, giving a meiotic index of about 50%. Sperm length was 12 to 30 $\mu$ .

WBPH chromosomes were holocentric. The kinetochores were diffused along the entire length of both autosomes and sex chromosomes. The diploid number was 2n=29, and 2n=30 in females. The sex chromosome in each testicular cell was a univalent X body, 2 $\mu$  long and 1 to 1.5 $\mu$  wide. The oogonial cells had XX bivalents,



1. Uninucleated (top) and binucleated (bottom) meiocytes (1000X) of *S. furcifera*



2. Complete genome in male *S. furcifera* (2n = 14 $\pi$  A + X). Sex chromosome is indicated by an arrow.