Effects of killing, fixing and mounting methods on taxonomic characters of parthenogenetic adult female *Caenorhabditis elegans* (Nematoda : Rhabditidae)

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SUMMARY

A comparative study of several commonly-used killing, fixing and mounting techniques was made to identify the optimum procedure for preparation of parthenogenetic adult female *Caenorhabditis elegans* for taxonomic examination. Nematodes were either *i*) killed in hot water (95 °C) and fixed in cold fixative (22 °C) or *ii*) killed and fixed in hot fixative by Seinhorst's (1966) technique. Both the qualitative and quantitative effects of five fixatives (FA 4:1; FP 4:1; TAF; FG and FAA) on each of twenty body characteristics were studied in temporary mounts of specimens killed by each method. Although all the fixatives caused significant shrinkage and/or distortion, killing and fixing in hot TAF (95 °C) produced the least-affected specimens. Subsequent processing of hot TAF-killed and -fixed specimens to lactophenol or glycerol caused further significant shrinkage and/or distortion. Of the four techniques studied, processing of nematodes to glycerol by the slow method affected fewest parameters and produced the most acceptable specimens.

RÉSUMÉ

Effets sur les caractères taxonomiques de la femelle parthénogénétique adulte de Caenorhabditis elegans *(Nematoda : Rhabditidae) des méthodes utilisées pour tuer, fixer et monter les nématodes*

Il a été procédé à une étude comparative des méthodes courantes utilisées pour tuer, fixer et monter les nématodes, étude ayant pour but de déterminer la procédure optimale de préparation de femelles parthénogénétiques adultes de *Caenorhabditis elegans* en vue de recherches taxonomiques. Les nématodes ont été *i*) tués dans l'eau chaude (95 °C) et fixés à froid (22 °C) ou *ii*) tués et fixés

ing to Seinhorst (1966). Hot water-killed, unfixed nematodes served as controls. The following fixatives were used : (*a*) FA 4:1 (10 ml 40 % formaldehyde, 1 ml glacial acetic acid, 89 ml distilled water); (*b*) FP 4:1 (10 ml 40 % formaldehyde, 1 ml propionic acid, 89 ml distilled water); (*c*) TAF (7 ml 40 % formaldehyde, 2 ml triethanolamine, 91 ml distilled water); (*d*) FG (8 ml 40 % formaldehyde, 2 ml glycerol, 90 ml distilled water); (*e*) FAA (6 ml 40 % formaldehyde, 20 ml 95 % ethanol, 1 ml glacial acetic acid, 40 ml distilled water).

Water and fixatives were contained in separate glass test tubes and were maintained at 95 °C in a water bath. Selected specimens, placed in a very small drop of saline (0.9 % w/v sodium chloride) in a glass cavity block, were flooded with 4 ml of hot water, or fixative, to ensure rapid death and fixation of nematodes. For cold fixation of nematodes, excess water from the cavity blocks containing hot water-killed nematodes was removed and the specimens were flooded with 4 ml of cold fixative (22 °C). Temporary mounts of nematodes (i.e. in water or fixative) were prepared using pieces of agar (2 g agar, Oxoid Ltd.; 50 mg $CuSO_4.7H_2O$; 100 ml distilled water) as cover-glass supports (Grewal, 1990) and were sealed with glyceel. Hot water-killed, unfixed nematodes were examined for taxonomic details and were measured immediately after killing whereas the fixed specimens were maintained at 22 °C for 10 days and then studied.

Processing to mounting medium

Nematodes were killed and fixed in hot TAF and, after 10 days in fixative at 22 °C were processed to lactophenol or glycerol using one of the following techniques : (*a*) Rapid lactophenol method (Franklin & Goodey, 1949); (*b*) Rapid method to glycerol (Baker, 1953); (*c*) Glycerol-ethanol method (Seinhorst, 1959); (*d*) Slow method to glycerol (Goodey, 1963). Permanent mounts were also prepared using agar pieces as cover-glass supports and were sealed with glyceel.

Data recording and analysis

A compound microscope was used for observations of both qualitative and quantitative effects. Drawings and measurements were made on twenty individual nematodes in each treatment using differential interference contrast optics. Observations on twenty different body parameters (Table 1), most of which are commonly used in rhabditid taxonomy (Andrássy, 1983) were made. Taxonomic ratios including, a, b, c, c', V, m (promesostom length/promesostom width) and t (distance from posterior flexure of gonad to tail end as a percentage of body length) were calculated and a two-way analysis of variance was made of the data recorded. Observations on qualitative features including the appearance of cuticle and hypodermis and the clarity of structures such as the nerve ring, glottoid apparatus, œsophageal collar and excretory duct were made using high magnification and oil immersion objectives.

Table 1

Effects of killing and fixing methods on morphometrics of adult female *C. elegans* : analysis of variance

Body parameters	*Significance*		
	HF	*CF*	*HF × CF*
A : LINEAR PARAMETERS			
1. Body length	**	***	*
2. Oesophagus length	**	***	***
3. Promesostom length	NS	***	***
4. Length from anterior end to œsophageal valve	*	***	***
5. Length from anterior end to vulva	**	***	***
6. Gonad length (anterior to posterior flexure)	NS	***	**
7. Length from posterior flexure of gonad to tail end	**	**	***
8. Tail length	NS	***	NS
B : WIDTH PARAMETERS			
1. At median bulb	**	**	*
2. At posterior bulb	***	***	***
3. At vulva	*	***	***
4. At anus	**	**	NS
5. Promesostom width	***	NS	*
C : TAXONOMIC RATIOS			
1. a (= body length/greatest body width)	NS	***	***
2. b (= body length/œsophagus length)	NS	***	***
3. c (= body length/tail length)	*	NS	***
4. c' (= tail length/body width at anus)	**	**	*
5. V (= length from anterior end to vulva as percentage of body length)	NS	**	***
6. m (= promesostom length/promesostom width)	***	***	*
7. t (= length from posterior flexure of gonad to tail end as percentage of body length)	NS	***	***

HF = killed and fixed in hot fixative (95 °C); CF = killed in hot water (95 °C) and fixed in cold fixative (22 °C); HF × CF = interaction between hot and cold fixatives; NS = not significant; * $p < 0.05$ ** $p < 0.01$ *** $p < 0.001$.

Results

KILLING AND FIXING

Quantitative effects

The effects of methods of killing and fixing on certain dimensions of adult female *C. elegans* are summarised in Figs 1, 2 and 3. Neither of the methods of preparation involving the use of fixatives produced nematodes that were similar to those killed in hot water and unfixed. All fixatives caused significant ($p < 0.05$) shrinkage in most characters measured and this was particularly marked with regard to : body length, length from anterior end to vulva (Fig. 1) and anal body width (Fig. 2). Most of the commonly-used taxonomic ratios that were considered were also significantly ($p < 0.05$) altered (Fig. 3).

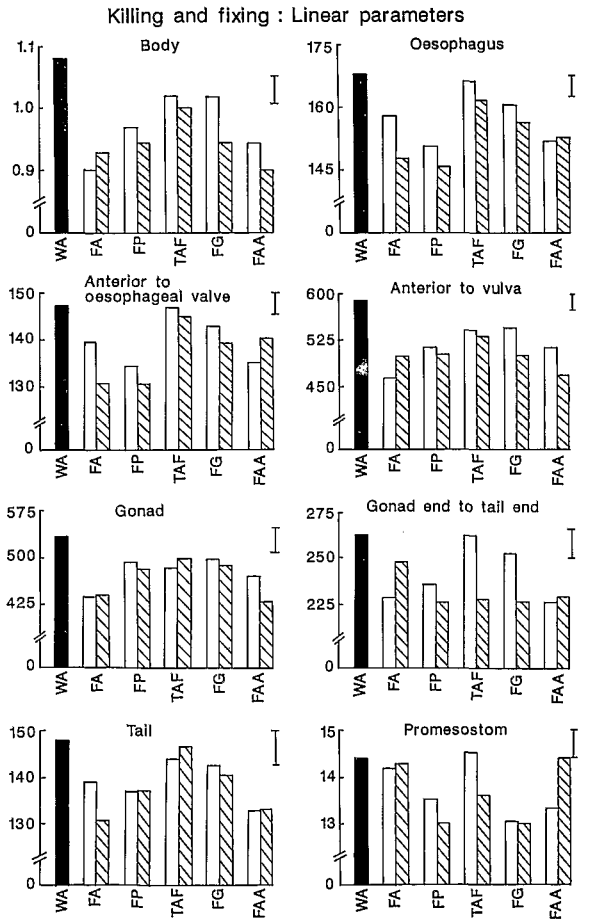


Fig. 1. Effects of killing and fixing methods on linear dimensions of parthenogenetic adult female *C. elegans*. For a key to methods refer to Fig. 2 and for the fixatives see " Materials and methods ". Bars represent L.S.D. ($p < 0.05$). All body measurements are in µm except body length (mm).

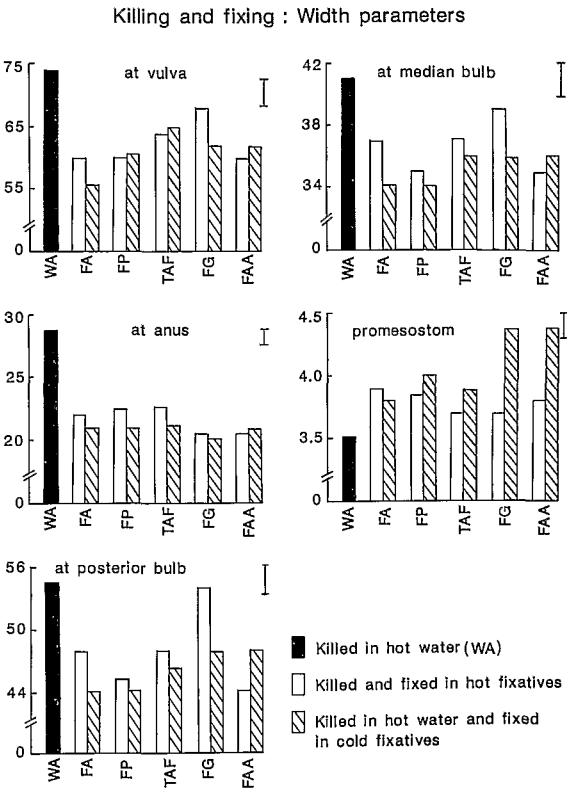


Fig. 2. Effects of killing and fixing methods on width parameters of parthenogenetic adult female *C. elegans*. For key to the fixatives see " Materials and methods ". Bars represent L.S.D. ($p < 0.05$). All body measurements are in µm.

Killing and fixing in hot fixative gave better results overall than killing with hot water and fixing in cold fixative (Table 1). Out of the 20 parameters evaluated, the former process had significant ($p < 0.05$) adverse effects on thirteen of them whereas the latter process significantly ($p < 0.05$) affected eighteen characters. Interactions between the two methods were significant for most characters indicating that a particular fixative had different effects when used hot or cold.

TAF significantly ($p < 0.05$) affected the fewest parameters. Eleven parameters were affected when nematodes were killed and fixed in hot TAF and fourteen were altered when the specimens were killed in hot water and fixed in cold TAF (Figs 1, 2, 3). The greatest changes were found with FP 4:1 and FAA : each fixative significantly ($p < 0.05$) affected eighteen parameters when specimens were killed and fixed in hot fixative and nineteen parameters when hot water-killed nematodes were fixed in cold fixative.

Qualitative effects

Cold and hot fixation in FA 4:1, FP 4:1, FG or FAA caused considerable swelling of the cuticle, and in

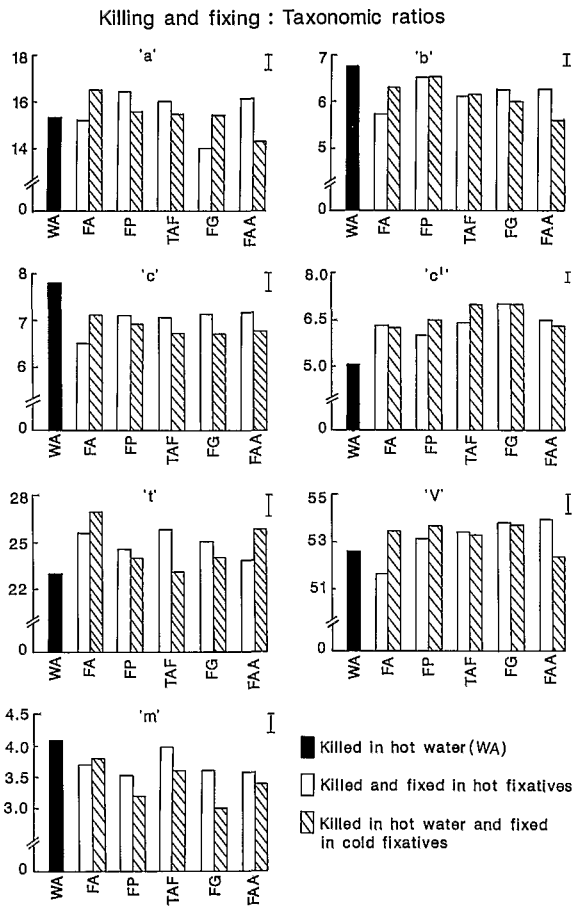


Fig. 3. Effects of killing and mounting methods on taxonomic ratios (see Table 1 for details) of parthenogenetic adult female *C. elegans*. For key to the fixatives see " Materials and methods ". Bars represent L.S.D. ($p < 0.05$).

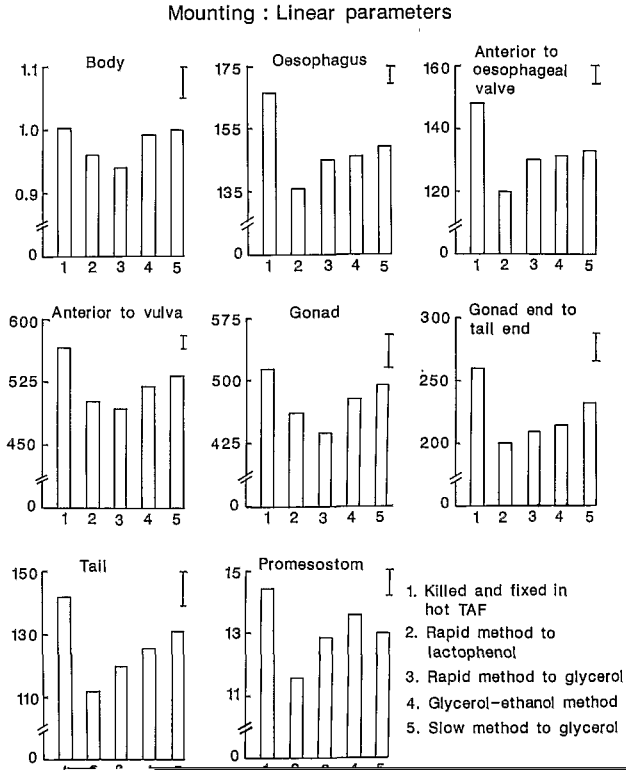
extreme cases resulted in the cuticle tearing away from the hypodermis, distortion of the hypodermis and darkening (browning) of the specimens. However, TAF caused clearing of the specimens and improved the appearance of most features. Formalin-glycerol fixative (FG) had the most pronounced effects on cuticle thickness and this was reflected in mean width of the specimens (Fig. 2). TAF caused no such qualitative distortion and produced the most acceptable specimens. Structures including the nerve ring, promesostom, glottoid apparatus (metastom), denticles and tri-radiate œsophageal valve were most distinct in TAF-fixed specimens (observed after 10 days in fixative) but the excretory duct and œsophageal collar were more distinct in all other fixatives.

the optimal method (i.e. in hot TAF) all subsequent processing methods caused significant shrinkage ($p < 0.05$) in most characters measured (Figs 4, 5, 6). Characters including the lengths of promesostom, œsophagus, and tail which were not significantly ($p < 0.05$) affected by the killing and fixing in hot TAF were affected adversely by all the mounting methods. Processing of nematodes to glycerol by the slow method had significant ($p < 0.05$) adverse effects on the fewest parameters — 13 out of 20 studied. The rapid lactophenol method significantly ($p < 0.05$) affected the most parameters — 17 out of 20 evaluated.

Table 2 lists the dimensions (including mean ± standard error and range) of adult female *C. elegans* when hot TAF-killed and -fixed specimens were processed to mounting medium (lactophenol or glycerol) by four different techniques.

Qualitative effects

Both the rapid lactophenol method and the rapid method to glycerol caused the greatest distortion of specimens and the loosening of cuticle away from the hypodermis. Although maximum distortion occurred in the region of the œsophagus the effects extended to the



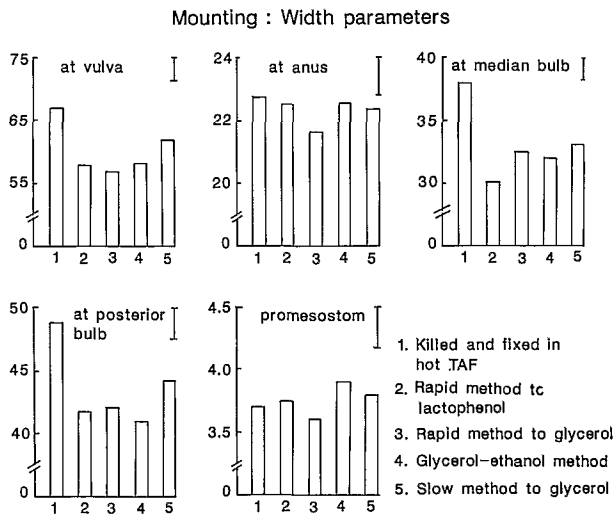


Fig. 5. Effects of mounting methods on width parameters of parthenogenetic adult female *C. elegans*. Bars represent L.S.D. ($p < 0.05$). All body measurements are in μm.

vulval region. The slow method to glycerol did not cause any such cuticular distortions. The glycerol-ethanol method (Seinhorst, 1959) also produced acceptable specimens.

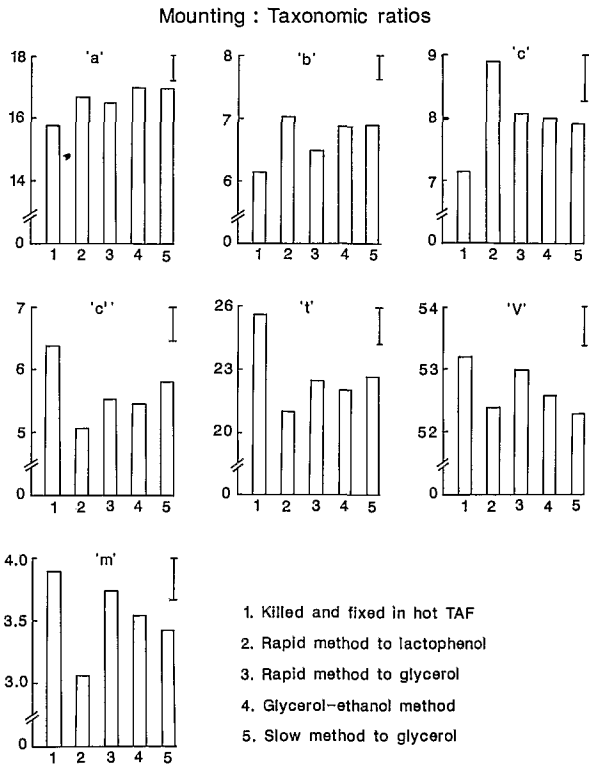


Fig. 6. Effects of mounting methods on taxonomic ratios (see

Table 2

Dimensions of parthenogenetic adult female *C. elegans* killed and fixed in hot TAF (95 °C) and processed to mounting medium by four different techniques

Parameters	*Mounting methods*			
	Rapid method to lactophenol	*Rapid method to glycerol*	*Glycerol-ethanol method*	*Slow method to glycerol*
Body length (µm)	957 ± 22* 728 — 1 070**	937.2 ± 18 734 — 1 015	984.3 ± 8.7 900 — 1 037	1 016 ± 12 878 — 1 097
Ratio a	16.7 ± 0.39 13.4 — 19	16.5 ± 0.26 13.8 — 17.9	17.0 ± 0.19 15.6 — 18.5	17.0 ± 0.29 14.9 — 19.6
Ratio b	7.0 ± 0.14 5.7 — 7.8	6.5 ± 0.12 5.6 — 7.8	6.7 ± 0.06 6.2 — 7.3	6.8 ± 0.11 6.1 — 7.8
Ratio c	8.8 ± 0.37 6 — 12.5	8.1 ± 0.32 7 — 12.5	8.0 ± 0.14 7 — 8.9	7.9 ± 0.13 7 — 9.2
Ratio c′	5.7 ± 0.22 3 — 6.6	5.6 ± 0.23 3 — 7.2	5.5 ± 1.0 4.4 — 6.2	5.8 ± 0.11 5.0 — 6.8
Ratio V	52.4 ± 0.44 49.5 — 57.5	53 ± 0.74 44.6 — 62.8	52.6 ± 0.36 47.3 — 54.9	52.3 ± 0.39 49.5 — 54.6

* Mean ± standard error
** Range

shrinkage sometimes produces ratios that have no com- characters of a ratio and thereby affects the ratios

[redacted]

body length and mean spicule length for *Hydromermis conopophaga* has been reported to be 44 (Poinar, 1968); 35 (Mulvey & Nickle, 1978) and 64 (Hominick & Welch, 1971).

Length and width of stoma are important characters in rhabditid taxonomy (Andrássy, 1983). Our observations revealed, however, that processing methods are liable to alter these parameters. For instance, the mean length and breadth of the promesostom ranged from

Mounting techniques may alter the specimens to the extent that processing artifacts may sometimes be interpreted as taxonomic characters. For example, *R. culicivorax* is morphologically similar to *R. iyengari* and has been distinguished primarily by the more acute angle of the papillary tract to the œsophagus and a tendency for a thinner cuticle and egg-shell (Ross & Smith, 1976). However, Curran and Hominick (1981) have concluded that the above characteristics are processing artifacts

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