

## Strain-specific variation in the pattern of caudal papillae in *Caenorhabditis briggsae* (Nematoda: Rhabditidae); implications for species identification

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*Caenorhabditis briggsae* is a member of the 'elegans group', a monophyletic clade of seven species within *Caenorhabditis* (Sudhaus & Kiontke, 1996). Species identifications within this group are difficult. *C. briggsae* and *C. elegans* can be distinguished in that they are hermaphroditic whereas other members of the *elegans* group are gonochoristic (Maupas, 1900; Nigon & Dougherty, 1949; Sudhaus & Kiontke, 1996). *C. briggsae* and *C. clavopapillata* can be distinguished from other *elegans* group members based on their patterns of caudal papillae (Kreis & Faust, 1933; Nigon & Dougherty, 1949; Friedman *et al.*, 1977).

Despite the general utility of caudal papillae patterns as diagnostic characters, their use to discriminate between *C. briggsae* and *C. elegans* came into question when a strain of *C. briggsae*, PB800, was obtained that displayed an *elegans* pattern at a high frequency. The canonical *C. briggsae* arrangement of caudal papillae is a 2/4+3 pattern with the third and fourth pair frequently being fused together (Nigon & Dougherty, 1949; Fig. 1A). This pattern also was reported for a second *C. briggsae* strain, AF16 (Fodor *et al.*, 1983). The *C. briggsae* arrangement differs from those of *C. elegans* and *C. remanei* which have a 2+(1)+3+3 pattern (Maupas, 1900; Sudhaus, 1974; Baird *et al.*, 1994; Fig. 1B, C). Immediately after its establishment, 70% of PB800 males displayed an *elegans* pattern on both sides. With subsequent inbreeding, this frequency decreased but still remained relatively high (see below).

To address their use as diagnostic characters, caudal papillae patterns of AF16, PB800 and three additional *C. briggsae* strains, HK104, HK105, and VT847, were determined by microscopic observations using differential interference contrast optics (magnification 400×). These strains were established from collections at disparate lo-

cations and should each represent a genetically distinct population (Table 1). Identifications of PB800, HK104, HK105, and VT847 as *C. briggsae* were confirmed by mating tests with AF16 (data not shown). These tests were scored as positive if the frequency of males approached 50% in the F1 generation. In the absence of mating, male frequencies resulting from self-fertilisation are less than 1% (Nigon & Dougherty, 1949). For PB800, additional mating tests with AF16 were conducted with sperm-depleted hermaphrodites (Baird *et al.*, 1992). These mating tests were scored as positive if any F1 progeny were obtained.

Among and within the *C. briggsae* strains AF16, PB800, HK104, HK105 and VT847, considerable variation in caudal papillae pattern was observed (Figs 1, 2). In AF16 and VT847, 2/4+3 patterns predominated with more than 85% of males exhibiting this *briggsae* pattern on both sides. (Expression of caudal papillae patterns on left and right sides was independent, hence the frequency of males exhibiting a single pattern on both sides was equal to the square of the overall frequency of that pattern.) In PB800, HK104, and HK105, frequencies of 2/4+3 and 2+(1)+3+3 patterns were approximately equal. In these strains, approximately 25% of males exhibited a *briggsae* pattern on both sides and approximately 25% an *elegans* pattern on both sides. Among the observed 2/4+3 patterns, the frequency with which caudal papillae 3 and 4 were fused also varied from strain to strain. This variation was correlated with the overall frequency of 2/4+3 patterns; fusions of caudal papillae 3 and 4 were most frequent in AF16 and VT847 and least frequent in PB800, HK104, and HK105. Other patterns also were observed at lower frequencies in PB800 and HK105. These included a 2+(2)+2+3 pattern, a 'sixless' pattern in which caudal papillae assumed a cylindrical morphology and was displaced anteriorly (see

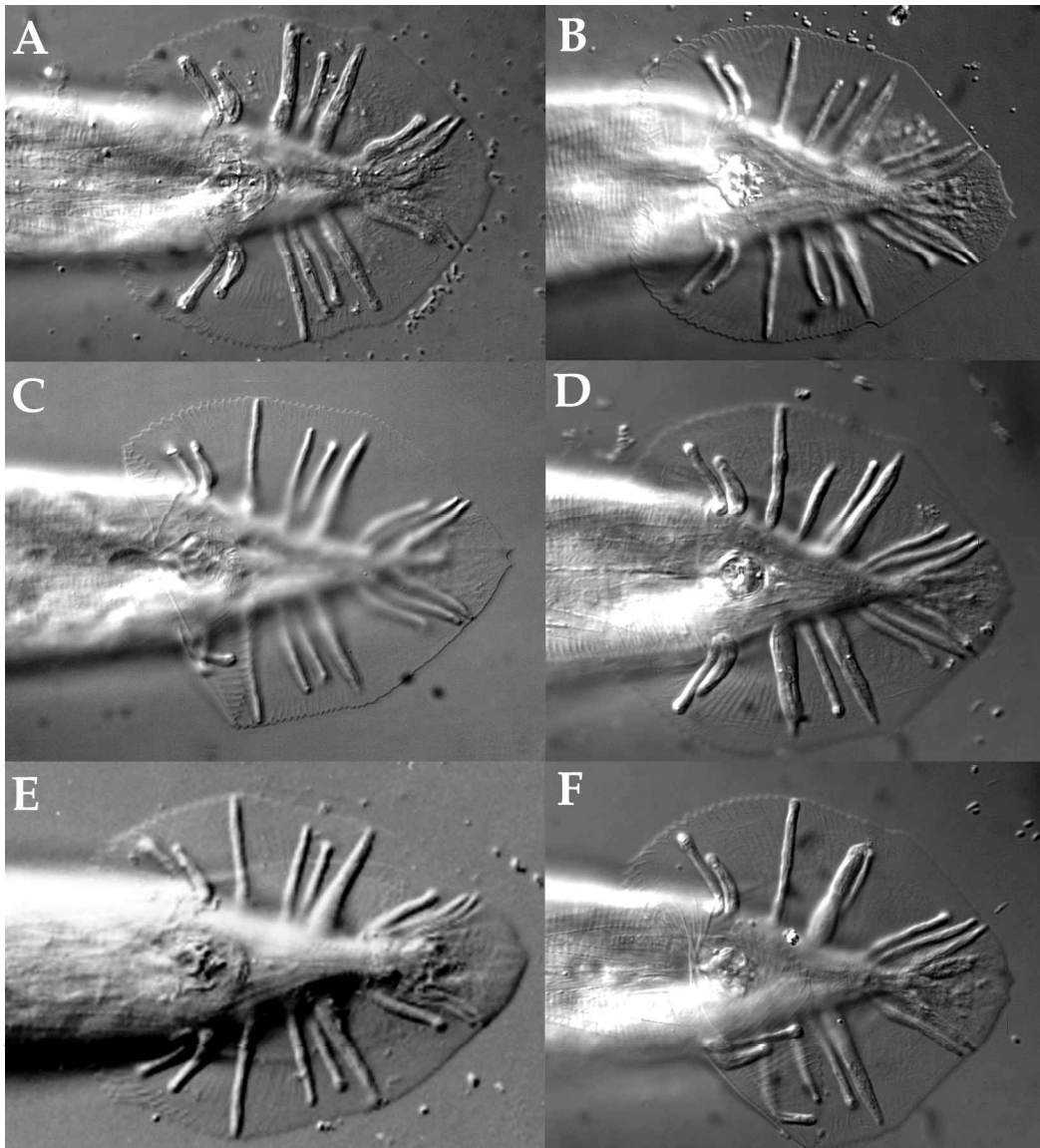
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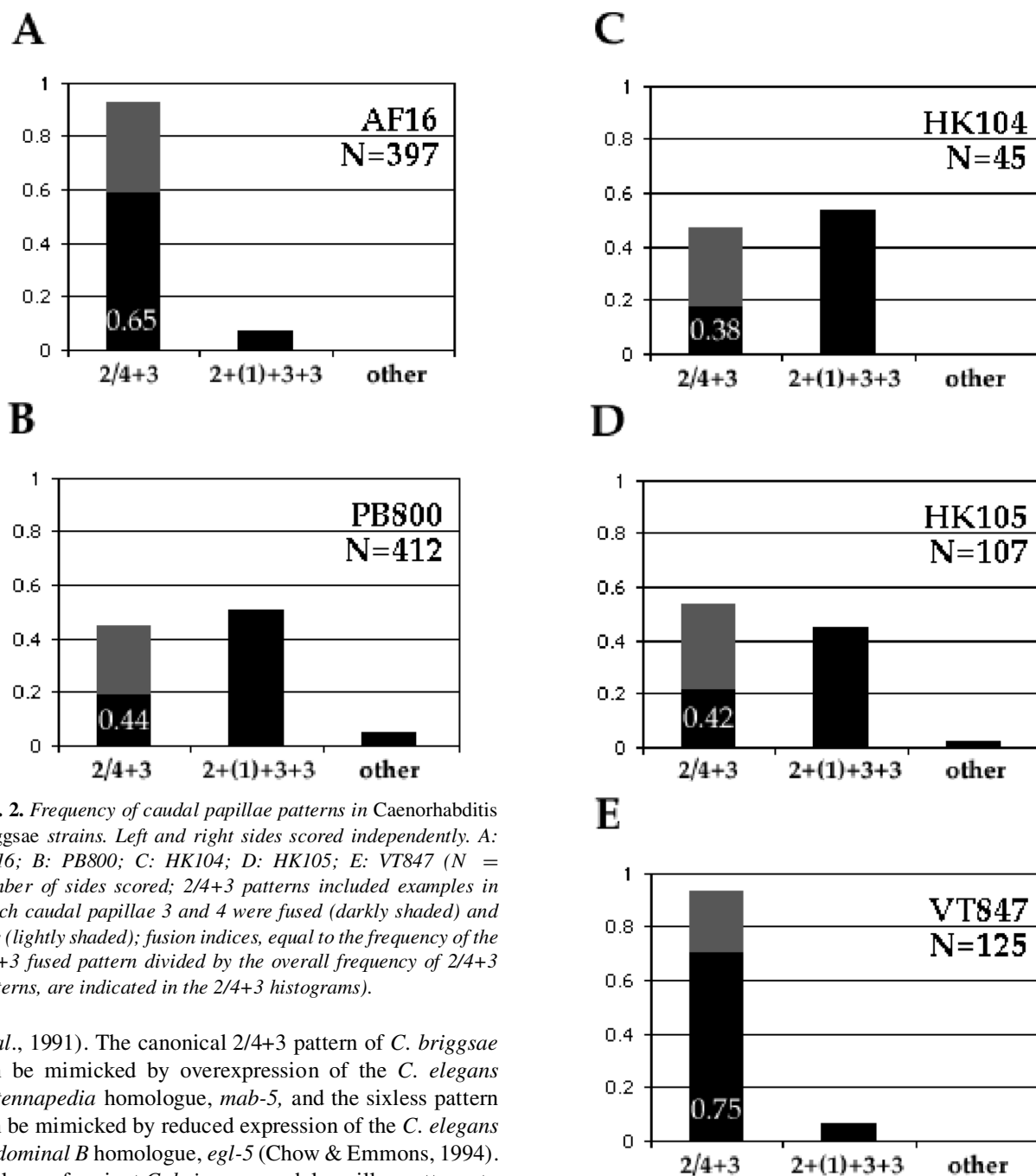


**Fig. 1.** Patterns of caudal papillae in *Caenorhabditis* species and variant patterns in *C. briggsae*. All micrographs are shown from a ventral aspect with the left side of each worm facing up. A: *C. briggsae* AF16 showing the canonical 2/4+3 pattern with papillae 3 and 4 fused on the left side and free on the right side; B, C: *C. remanei* EM464 and *C. elegans* N2, respectively, both showing the 2+(1)+3+3 pattern that is typical for these species; D-F: Variant patterns of caudal papillae in *C. briggsae* PB800 males (in D, a 2+(1)+3+3 pattern, both sides, in E, a 2+(1)+3+3 pattern, left side, and a 2/4+3 pattern, right side and in F, a modified 2+(1)+3+3 with papilla 6 displaced anteriorly and fused with papilla 4, left side, and an unmodified 2+(1)+3+3 pattern, right side).

Fig. 1F), and patterns in which various caudal papillae were absent.

These results demonstrate that caudal papillae pattern, as a diagnostic character to discriminate between *C. briggsae* and *C. elegans*, must be used with caution. This is especially true as *C. briggsae* and *C. elegans* are her-

maphroditic; observations of the limited number of males typically available in natural populations could easily lead to a misdiagnosis. The observed variation in *C. briggsae* caudal papillae pattern also provides an opportunity to investigate the evolution of this character. Caudal papillae pattern is sensitive to mutation in several genes (Baird



**Fig. 2.** Frequency of caudal papillae patterns in *Caenorhabditis briggsae* strains. Left and right sides scored independently. A: AF16; B: PB800; C: HK104; D: HK105; E: VT847 ( $N$  = number of sides scored; 2/4+3 patterns included examples in which caudal papillae 3 and 4 were fused (darkly shaded) and free (lightly shaded); fusion indices, equal to the frequency of the 2/4+3 fused pattern divided by the overall frequency of 2/4+3 patterns, are indicated in the 2/4+3 histograms).

*et al.*, 1991). The canonical 2/4+3 pattern of *C. briggsae* can be mimicked by overexpression of the *C. elegans* *Antennapedia* homologue, *mab-5*, and the sixless pattern can be mimicked by reduced expression of the *C. elegans* *Abdominal B* homologue, *egl-5* (Chow & Emmons, 1994). Linkage of variant *C. briggsae* caudal papillae patterns to the *C. briggsae* homologues of *mab-5* and *egl-5* could be used to test whether or not character evolution of the *C. briggsae* caudal papillae pattern results from molecular evolution in the *C. briggsae* *HOM-C/Hox* cluster.

Regardless of the utility of caudal papillae pattern as a diagnostic character, the species status of *C. briggsae* is not in question. *C. briggsae* is reproductively isolated

**Fig. 2.** (Continued).

and phylogenetically distinct from *C. elegans* (Nigon & Dougherty, 1949; Friedman *et al.*, 1977; Thomas & Wilson, 1991; Baird *et al.*, 1992; Fitch *et al.*, 1995; Baldwin *et al.*, 1997).

**Table 1.** Source of *Caenorhabditis briggsae* strains.

Strain <sup>a)</sup>	Locale	Source	Ref.
AF16	Gujarat, India	Soil	Fodor <i>et al.</i> , 1988
HK104	Okayama, Japan	Mushroom	H. Kagawa, pers. comm.
HK105	Sendai, Japan	Mushroom	H. Kagawa, pers. comm.
PB800	Dayton, OH, USA	Mushroom	S.E. Baird, unpubl.
VT847	Haena, Kauai, HI, USA	Soil	V. Ambros, pers. comm.

<sup>a)</sup> AF16 was confirmed as *C. briggsae* by mating tests with DH1300, a strain derived from the original isolate of *C. briggsae* (Fodor *et al.*, 1983). All other strains were confirmed as *C. briggsae* by mating tests with AF16.

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