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SHORT COMMUNICATION

STRUCTURAL VARIABILITY OF AN IDENTIFIED INTERNEURONE IN LOCUSTS FROM A WILD POPULATION

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One property of invertebrate nervous systems used to great experimental advantage is the 'identifiability' of individual neurones. A principal tenet of the 'identified neurone' concept is that a certain degree of structural regularity can be expected when investigating an identified cell in individual members of a single species. Yet in organisms that reproduce sexually some degree of structural variability should be expected over and above that attributed to developmental noise. Genotypic lineage appears to be a strong determinant of neurone structure. Recent studies using controlled lineages through selective and parthenogenetic breeding have indicated a fairly strong genetic influence on neurone number, structure and interconnections (Macagno, Lopresti & Levinthal, 1973; Goodman, 1977, 1978; Steeves & Pearson, 1983). In wild populations, however, some genetic variability may be selectively eliminated through environmental factors as well as intra- and interspecific biological interactions.

In the locusts, *Schistocerca gregaria* and *Locusta migratoria*, the metathoracic branching pattern of a single visual interneurone, the descending contralateral movement detector (DCMD), has been found to be highly variable (Pearson & Goodman, 1979). The specimens used in this investigation came from a long-standing and presumably highly inbred laboratory colony. Moreover, DCMD structure and synaptic connectivity in individuals of isogenic clones are less variable than in sexually reproduced individuals, and show clone-specific structural differences, but still with significant variability within clones (Steeves & Pearson, 1983). In this paper, the description of DCMD branching structure in individuals of a related species, *Schistocerca albolineata*, collected from a wild population, indicates that metathoracic branching variability, particularly in contralateral ventral and abdominal branches, is less than in laboratory-reared sexually reproduced *Schistocerca* and *Locusta* species (Pearson & Goodman, 1979).

Male and female *S. albolineata* were collected in Millsite Canyon, Superstition Mountains, just east of Apache Junction, Arizona in August and September. They were maintained in laboratory cages containing fresh water, lettuce and wheat germ. For intracellular recording and dye injection, animals were opened dorsally, and the metathoracic ganglion was prepared according to the methods of Pearson & Goodman (1979). Microelectrodes (10–30 MΩ when filled with 3 mol l^{-1} KCl) were

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tip-filled with a 4% solution of Lucifer Yellow CH (Stewart, 1978), and back-filled with 1 mol l^{-1} LiCl. DCMD neurones were penetrated at the point of axon entry into the metathoracic ganglion, and identified by the discharge of high frequency spikes in response to a movement in the contralateral visual field. Dye-injection involved passing 2–8 nA negative current pulses of 500 ms duration at 1 s^{-1}. Following injection, the mesothoracic, metathoracic and first free abdominal ganglia were dissected from the animal, fixed for 2 h in 3% formaldehyde in locust saline, dehydrated and cleared in methyl salicylate. DCMD neurones from 21 animals were scored according to the presence or absence of five major branches that have been previously described: anterior, ipsilateral ventral, contralateral ventral, dorsal and abdominal (Pearson & Goodman, 1979).

The basic metathoracic branching structure of DCMD interneurones of *S. albolineata* is similar to that of *S. gregaria* and *L. migratoria* in that the five major axonal branches can be easily identified (Fig. 1). As shown in Table 1, variability was very low for all but one branch, the dorsal branch of *S. albolineata* DCMDs, which was lacking in nearly one-third of the animals. In comparison with similar data from inbred laboratory colonies, in which variability was found in all branches (Table 1), overall variability in *S. albolineata* is considerably lower. For example, it is reported that in *S. gregaria* and *L. migratoria* only 23% of the animals had a full complement of branches and 35% had more than one branch missing. In *S. albolineata*, 57% of the animals had all five branches, and none were observed to be missing more than one branch.

A breakdown of branching pattern according to sex revealed an overall similarity in branching variability in males and females (Table 1). For example, in pooled

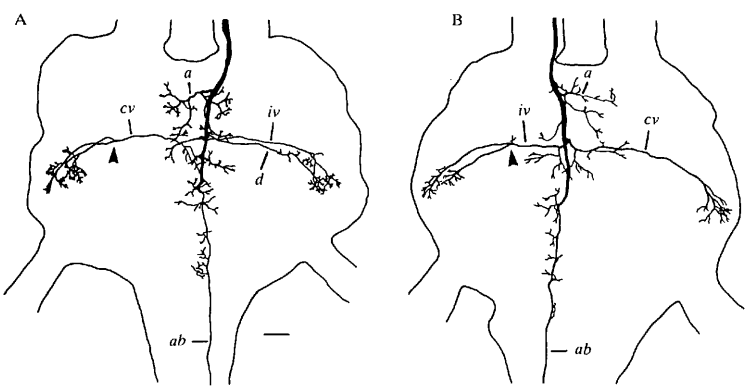

Fig. 1. Branching of the DCMD interneurone in the metathoracic ganglion of *Schistocerca albolineata*. (A) Branching pattern showing full complement of major branches: *a*, anterior branch; *d*, dorsal branch; *iv*, ipsilateral ventral branch; *cv*, contralateral ventral branch; *ab*, abdominal branch. Note the bifurcation in the contralateral ventral branch (arrowhead). (B) Branching pattern showing the most frequent branch omission, the dorsal branch. Note the bifurcation in the ipsilateral ventral branch (arrowhead). Calibration, 100 μm.

Table 1. *Variability of DCMD axonal branching pattern within the metathoracic ganglion of* Schistocerca albolineata

Number of animals	Anterior branch	Ipsilateral ventral branch	Contralateral ventral branch	Dorsal branch	Abdominal axon
12	+	+	+	+	+
6	+	+	+	−	+
2	+	+	+	+	−
1	+	−	+	+	+
Females					
13	13	12	13	10	12
	(100%)	(92·3%)	(100%)	(76·9%)	(92·3%)
Males					
8	8	8	8	5	7
	(100%)	(100%)	(100%)	(62·5%)	(87·5%)
Total					
21	21	20	21	15	19
	(100%)	(95·2%)	(100%)	(71·4%)	(90·5%)
Inbred population					
26	25	24	19	18	15
	(96·2%)	(92·3%)	(73·1%)	(69·2%)	(57·7%)

In the top of the table a breakdown of branching patterns of individual animals is given (+ = branch present, − = branch missing). Compilation of these data appears in the lower part of the table (expressed as number of animals with branch present) and includes a breakdown according to sex.
Data from the inbred population is from Pearson & Goodman (1979).
Numbers in parentheses refer to the percentage occurrence of a branch.

data, the branch with the most variability, the dorsal branch, was missing in 29% of the DCMD fills. Of this total, 23% of females and 37% of males lacked this branch. This difference was not found to be significant (based on the Log-Likelihood Ratio, $P < 0{\cdot}25$) with this small sample size.

Secondary branching was analysed in *S. albolineata* using an easily recognized feature of the ipsilateral and contralateral ventral branches. In two-thirds of the animals tested, one or both of the ventral branches had a major bifurcation in the proximal two-thirds of its length (Fig. 1). For scoring purposes, divisions farther along the branch were not counted since terminal branch arborizations occurred in all ventral branches. The major bifurcation was present in 20% of the ipsilateral ventral branches and 52% of the contralateral ventral branches. The degree of variability in this example of secondary branching is much greater than that exhibited in the primary branching structure of DCMD.

One explanation for the observed absence of axon branches in dye-filled neurones is that not all branches fill with dye. In this case, absence of branches would reflect dye-filling artifacts rather than true structural abberations. This is not the case for inbred animals since parallel electrophysiological experiments indicated that an absence of specific branches could be correlated with an absence of specific physiological connections (Pearson & Goodman, 1979). The reliability of Lucifer-fills used in this investigation has not been similarly tested, but any such artifact would imply that variability in wild animals is even less than reported, further underscoring differences in structural variability in the two populations.

Pearson & Goodman (1979) state that the variability in DCMD branching structure is so great that a normal pattern cannot be described. This is not the case for the DCMDs of *S. albolineata* in which two of the five major branches were present in all animals examined, and two others appeared in over 90% of the test animals. The only branch to show considerable variation was the dorsal branch, which has been shown in other species to make weak synaptic connections to flight motor neurones (Pearson & Goodman, 1979). Variability in this branch could easily reflect major species differences since this connection was found only rarely (15% occurrence) in *S. gregaria* but more frequently (78%) in *L. migratoria*. As pointed out previously (Steeves & Pearson, 1983), connections that produce significant behavioural contributions in known circuits, such as the anterior branch which provides relatively strong input to the escape-jump system (Pearson, Heitler & Steeves, 1980; Steeves & Pearson, 1982), show little variability. This branch was present in all *S. albolineata* examined.

Variability in the secondary branching pattern of DCMD in *S. albolineata* is considerably greater than that of the primary branching pattern. Similar results have been obtained previously in several preparations (i.e. Macagno *et al.* 1973; Goodman, 1978; Pearson & Goodman, 1979), and may reflect the level at which developmental noise overrides the genetic plan. With this in mind, a question can be raised concerning the source of phenotypic variability in inbred populations relative to wild populations. Is the variability due to increased genotypic variability, or to a relatively high tolerance for non-genetic modifications? Support for the latter idea can be drawn from work on isogenic locust clones (Steeves & Pearson, 1983): despite definite clone-related structural quirks, intra-clone variability was still noted. A possible explanation for the low variability in wild populations, then, might centre on a low tolerance for genetic and/or non-genetic modification of neurone structure. It is difficult to resist speculation about the potential role of intra- and interspecific interactions in controlling the degree of variability of the DCMD interneurone in wild populations, since its role in escape behaviour has been documented (Pearson *et al.* 1980). Foremost would be the importance of predation pressures if structural modification of this interneurone resulted in a change of escape ability. Movement detecting neurones might also play an important role in mate location or selection and thus directly influence reproductive success of the individual.

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