

The adult ventral nerve cord as a phylogenetic character in brachyceran Diptera

David K. Yeates*, David J. Merritt, Claire H. Baker

Department of Zoology and Entomology, University of Queensland, Brisbane, Australia

Received 12 November 2001 · Accepted 12 March 2002

Abstract

Insect ganglia are often composed of fused segmental units or neuromeres. We estimated the evolution of the ventral nerve cord (VNC) in higher Diptera by comparing the patterns of neuromere fusion among 33 families of the Brachycera. Variation within families is uncommon, and VNC architecture does not appear to be influenced by body shape. The outgroup pattern, seen in lower Diptera, is fusion of neuromeres belonging to thoracic segments 1 and 2 (T1 and T2), and fusion of neuromeres derived from T3 and abdominal segment 1 (A1). In the abdomen, neuromeres A7–10 are fused into the terminal abdominal ganglion (TAG). Increased neuromere fusion is a feature of the Brachycera. No brachyceran shows less fusion than the outgroups. We established six pattern elements: (1) fusion of T1 and T2, (2) fusion of T3 and A1, (3) fusion of the T1/T2 and T3/A1 ganglia, (4) increase in the number of neuromeres comprising the TAG, (5) anteriorward fusion of abdominal neuromeres, and (6) the complete fusion of thoracic and abdominal neuromeres into a synganglion. States 1 and 2 are present in the outgroup lower Diptera, and state 3 in the Xylophagomorpha, Stratiomyomorpha, Tabanomorpha and Cyclorrhapha. State 4 is a feature of all Eremoneura. State 5 is present in Cyclorrhapha only, and state 6, fusion into a synganglion, has evolved at least 4 times in the Eremoneura. Synapomorphies are provided for the Cyclorrhapha and Muscoidea, and a grouping of three basal brachyceran infraorders Xylophagomorpha, Stratiomyomorpha and Tabanomorpha. The patterns of fusion suggest that VNC architecture has evolved irreversibly, in accordance with Dollo's law.

Key words: Diptera, ventral nerve cord, neuromere, evolution, phylogeny

Introduction

Many aspects of the phylogenetic relationships of the Diptera remain poorly known (Yeates & Wiegmann 1999). For example, the relationships of infraorders of Brachycera are not readily apparent from comparisons of morphological characters (Woodley 1989, Stuckenberg 2001). Molecular data promise to provide new perspectives on these relationships, and studies of this nature have been published (Friedrich & Tautz 1997) and are underway (B. M. Wiegmann & V. Kulasekera, pers. comm. 2001). Although morphological data continue to be recognized as important in constructing phylogenies, there have been few recent attempts to incorporate novel character sets to address the outstanding questions. Shaw & Meinertzhagen's (1986) investigation of synaptic connectivity within the lamina and medulla provided 6 novel synapomorphies that are congruent with the accepted phylogeny of Diptera. For example, the Brachycera have

an apomorphic, asymmetrical pattern of photoreceptive rhabdomeres. In an elegant anatomical comparison of the dipteran gut, King (1991) found that variation in the structure of the stomodeal valve is a useful character in the Diptera. He found unambiguous state transformations of increased complexity, particularly in the elaboration of the muscoid cardia, defining the Schizophora and Muscoidea. More recently, anatomical details of the neural organization of the fly visual system (Buschbeck 2000) and reproductive system (Fritz 2002) have been studied. In general, these novel character systems show strong congruence with phylogenetic hypotheses based on traditional character systems. However, they also display evidence of homoplasy, usually in the form of reversals and convergence. The latter can be explained by adaptive shifts away from the patterns dictated by phylogeny. We believe there are many more informative characters and functional insights to be gleaned from detailed and systematic examination of morphological character systems.

*Corresponding author: David K. Yeates, CSIRO Entomology, PO Box 1700, Canberra ACT 2601, Australia; e-mail: david.yeates@csiro.au

Many of the unresolved areas of dipteran phylogeny represent very deep and fundamental nodes in their evolution. These nodes mark evolutionary events that probably occurred in the late Palaeozoic and early Mesozoic differentiation of dipteran lineages (Yeates & Wiegmann 1999). Very conservative character systems will be most informative for resolving these questions. Our aim here is to assess whether the state of neuromere fusion in the adult ventral nerve cord is an informative character for determining phylogenetic relationships among the main lineages of brachyceran Diptera.

The ventral nerve cord (VNC) of insects is comprised of the subesophageal, thoracic and abdominal ganglia. Ganglia are often composed of multiple, fused neuromeres. To avoid confusion we refer to the segmental units of the VNC as neuromeres, and refer to them as ganglia where they stand as single units or are fused with adjacent neuromeres. Although individual neuromeres of insects have a highly conserved internal structure of tracts and commissures (Leise 1991, Edwards & Palka 1991), great variability is evident in the degree of fusion of neuromeres to form compound ganglia. In extreme cases of fusion, the outlines of individual neuromeres are no longer visible (Boyan & Ball 1993), although the segmental units can still be distinguished internally through the repeating patterns of tracts or other structures (Tyrer & Gregory 1982) and by tracing the origin of nerves innervating the body wall.

The most comprehensive comparative analysis of VNC organization in Diptera so far was carried out over 100 years ago by Brandt (1879, reproduced in Fritz 2002) who established the VNC pattern in approximately 27 families of adult brachyceran Diptera (based upon currently accepted family designations). He found that in Brachycera the thoracic neuromeres are either completely fused or divided into two ganglia. The first abdominal neuromere is always fused with the third thoracic. Sometimes additional abdominal neuromeres are fused into the thoracic ganglion. The terminal abdominal ganglion (TAG) represents a fusion of a variable number of neuromeres. Overall, the largest variation occurs in the numbers of abdominal neuromeres fused into the thoracic ganglion, and in the number fused into the terminal abdominal ganglion. All ganglia between the TAG and thoracic ganglion are composed of single neuromeres.

No systematic attempt has been made to incorporate adult neuromere fusion into a modern synthesis of dipteran relationships. Recently, Melzer et al. (1995) examined the larval ventral nerve cord of 17 families of Diptera Brachycera, and described 4 levels of neuromere fusion. Their results indicate some degree of homoplasy in larval VNC ganglion structure in accordance with the currently accepted lower brachyceran phylogeny. It is obvious that ganglion structure of the larval VNC rarely matches that of the adult, as Brandt has shown.

In this study, we examine the pattern of neuromere fusion in a broad range of adult Brachycera (33 families) and describe 10 patterns of neuromere fusion, representing 6 more states than recorded in larvae (Melzer et al. 1995). We show that this character system in adults contains synapomorphies that are congruent with the currently accepted phylogeny. Our unique contribution is to show that the character system provides new insights into the relationships at the base of the Brachycera, a subject of recent debate.

Materials and methods

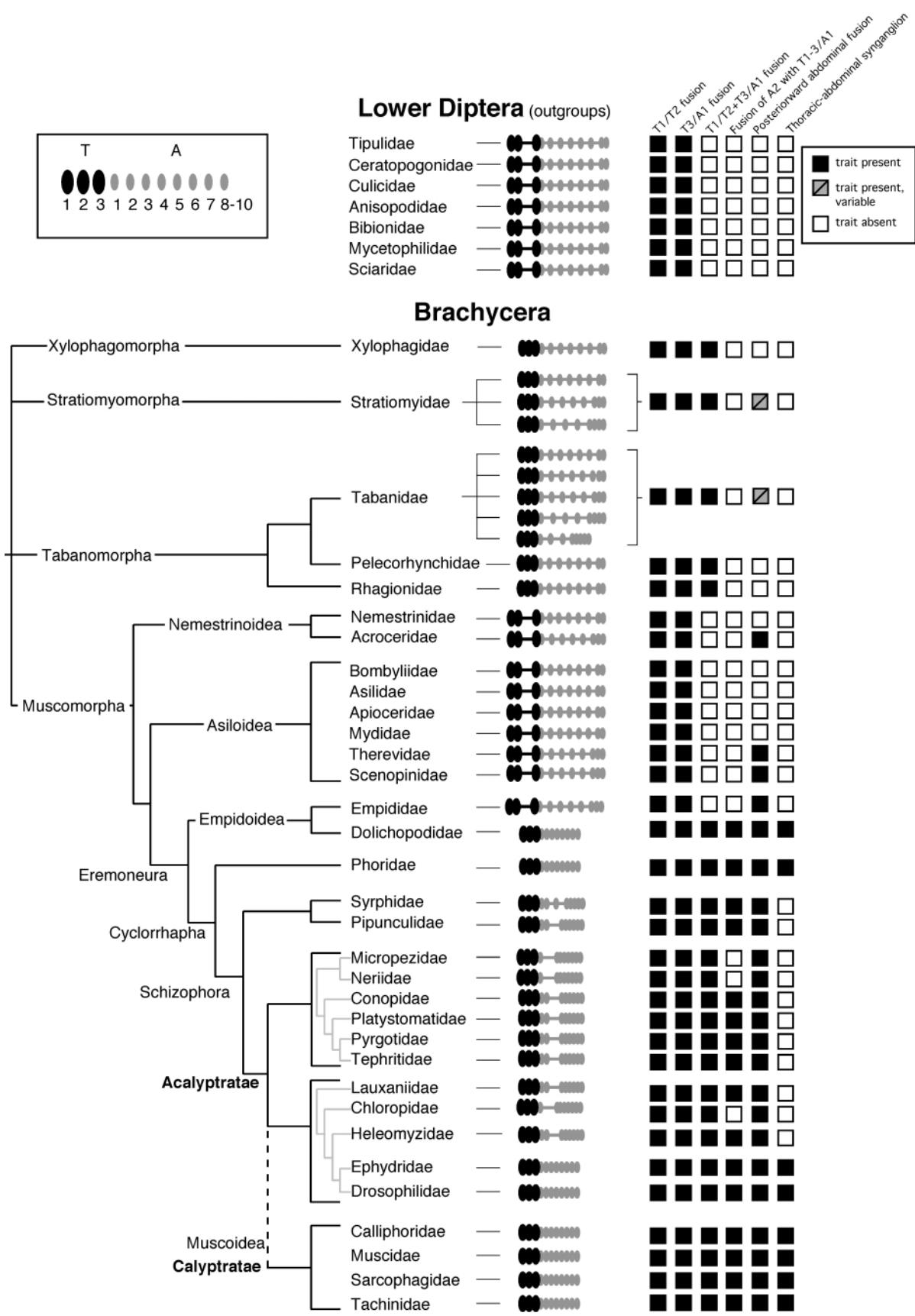
Specimens for this study were collected over the summers of 1996 to 1998 in south east Queensland. Individual flies were hand collected by members of the systematic entomology program at the University of Queensland, especially Jeff Skevington, Shaun Winterton, Chris Lambkin and DKY. Additional specimens were collected in an intensive malaise trapping survey conducted by Narelle Power. Specimens were identified by G. Daniels (Curator, University of Queensland Insect Collection, UQIC) and DKY. A complete list of the examined species is in the Appendix.

Adult flies, either fresh or fixed in 4% formaldehyde or 70–80% ethanol, were dissected under saline. Individuals were opened along the dorsal midline and the gut was removed to reveal the VNC. A few drops of 1:1 methylene blue, azure B solution in the dissecting solution temporarily stained the internal tissues to help recognition of fine nerve branches. The positions of ganglia in relation to the thoracic and abdominal segments were recorded. In addition, the segmental origin of each ganglion was determined by following the stained nerves to the segment they innervate. Dissected specimens were stored in 80% ethanol and vouchered in the UQIC.

Results

The broad pattern of VNC architecture we found is illustrated in Figure 1. The results are overlaid on our current understanding of brachyceran relationships, derived from the recent review by Yeates & Wiegmann (1999). We refer to the individual neuromeres by the segment corresponding to their embryonic derivation; thoracic segments T1–3 & abdominal A1–10. The posterior fusion of neuromeres is referred to as the terminal abdomi-

Fig. 1. Diagrammatic representations of ventral nerve cord (VNC) fusion in Diptera arranged phylogenetically according to Yeates & Wiegmann (1999). The left column is a schematic representation of the nerve cord (thoracic and abdominal neuromeres shown in different shades according to inset). The right hand column shows ventral nerve cord fusion interpreted in the form of character states. Derivation of character states from VNC fusion is explained in the text. The diagram includes only families covered in the present study.



nal ganglion (TAG). The complete fusion of all neuromeres into a single ganglion in the thorax is called the thoracic synganglion.

Outgroup pattern in lower Diptera

The outgroup pattern, seen in a variety of lower Diptera, is fusion of neuromeres belonging to T1 and T2, and fusion of T3 and A1. In the abdomen, ganglia A2–A6 are separate (i.e. comprised of individual neuromeres), and neuromeres A7–10 are fused into the TAG. We found this arrangement to be widely distributed in the Lower Brachycera, and in all putative sister-groups of the Brachycera; Psychodomorpha, Bibionomorpha and Tipulomorpha (Wood & Borkent 1989; Oosterbroek & Courtney 1995; Michelsen 1996). We therefore take this to be the outgroup pattern for the Brachycera.

Increased neuromere fusion is an advanced feature of Brachycera

In comparison to the VNC architecture of the outgroup lower dipteran families, several interesting features of the brachyceran VNC emerge. Firstly, no group in Brachycera shows less fusion than the lower dipteran outgroups. However, some basal muscomorphs belonging to the Nemestrinoidea and Asiloidea are identical to the pattern seen in the lower Diptera. Increased levels of fusion of abdominal ganglia are seen in the Eremoneura. This pattern is consistent with the notion that, in general, increased neuromere fusion is an advanced feature of the Brachycera.

A more complex pattern of fusion occurs in the thorax, where three basal infraorders of the Brachycera – the Xylophagomorpha, Stratiomyomorpha and Tabanomorpha – all share the advanced fusion of all three thoracic neuromeres and A1 into a single body located in the thorax. In contrast, the basal Muscomorpha (Nemestrinoidea and Asiloidea) all retain the level of fusion seen in the lower Diptera, with neuromeres T1 and T2 being fused but separate from the fused neuromeres T3 and A1.

Complete fusion of all neuromeres into a single synganglion occurs four times in the Eremoneura. It occurs in the Dolichopodidae (which are recognised as an advanced clade of Empidoidea), Phoridae, some acalyptates and in the Muscoidea. The structure of the synganglion shows some differences between Dolichopodidae and acalyptate and calyptrate flies. According to our current knowledge of brachyceran phylogeny, it is likely that each of these occurrences has evolved independently (Fig. 1).

The Pipunculidae and most acalyptates are unique in having just two ganglia: (1) a compound thoracic ganglion composed of T1–A1, plus in some cases A2; (2) a TAG composed of the remaining neuromeres. This form is phylogenetically intermediate between flies with a

number of discrete thoracic and abdominal ganglia and those with a single thoracic synganglion.

Variation within families is uncommon

Only two families, the Tabanidae and Stratiomyidae, show intrafamilial variation in VNC architecture. Members of the Tabanidae have VNC architecture that varies in the degree of fusion of the terminal abdominal neuromeres. Some, e.g. *Scaptia* Walker, have a pattern resembling the outgroup. Others, e.g. *Dasybasis* Macquart, show fusion of abdominal neuromeres A4–10. In Stratiomyidae there is also a variable degree of fusion of the abdominal neuromeres into the terminal abdominal ganglion. It seems that these groups are relatively plastic in the degree of fusion of abdominal neuromeres into the TAG.

Body shape and VNC architecture

To determine whether the pattern elements are the same within families we examined numerous members of the lower cyclorrhaphan (Aschiza) family Syrphidae and the Bombyliidae. In both groups a number of representative genera were available, including a variety of body forms. All members of each family proved to have the same pattern. Fig. 2 shows the conserved pattern of ganglion structure in two species pairs, one from each family, with extremely divergent body shapes. Bombyliids display an 8-ganglion pattern identical to the outgroup pattern shown in Fig. 1. Syrphids have a unique pattern of three ganglia, the first consisting of T1–3 + A1–2 in the thorax, the second being an isolated A3 ganglion, and the third ganglion representing the remaining abdominal neuromeres. Within each family, long-bodied and short-bodied representatives show the same VNC ganglion pattern. The location of ganglia within the body varies between species (Fig. 2A vs B), but the segmental origin of the ganglia remains the same. We found no evidence that body shape within a family influenced the pattern of ganglion fusion.

Discussion

Outgroup pattern

A string of isolated segmental ganglia is generally regarded as comprising the primitive condition of VNC organization (Bullock & Horridge 1965). There is a progressive tendency for higher degrees of fusion in more advanced insect taxa (Leise 1991). In the present study the outgroup thoracic pattern is the fusion of T1 and T2, and fusion of T3 and A1. In the abdomen, ganglia A2–A6 are comprised of individual neuromeres, and neuromeres A7–10 are fused into a TAG. Some other lower dipteran families have separate ganglia in the thorax and abdomen

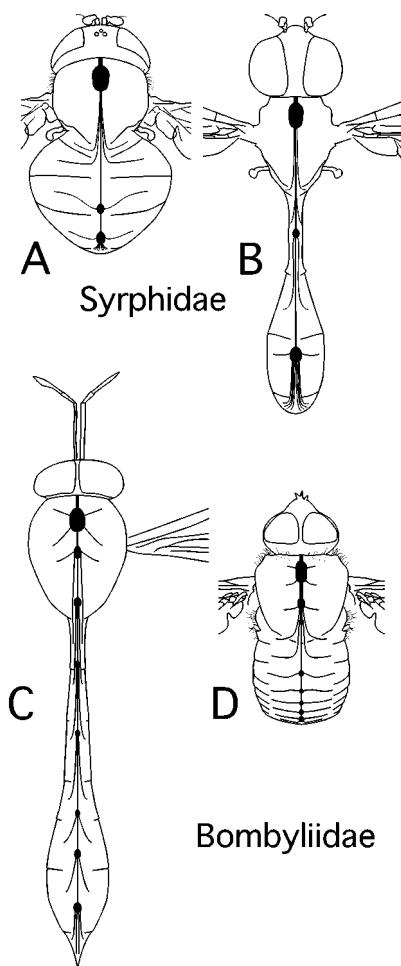


Fig. 2. Ventral nerve cord fusion in representatives of Syrphidae (A, B) and Bombyliidae (C, D). For each family, two species with widely divergent body shape have been chosen. In both cases, the family-specific arrangement of ganglia is preserved (refer to Fig. 1). A, B are shown at the same scale, different from the scale for C, D. Body shape is drawn to scale. The nerves and ganglia are drawn schematically, although the positions of ganglia within the body are drawn to scale.

anterior to the TAG. For example, the nymphomyiid fly, *Nymphomyia alba* Tokunaga shows complete separation of all neuromeres between T1 and A7 (Tokunaga 1936), reinforcing the view that progressive neuromere fusion is a characteristic of dipteran evolution.

Basal Brachycera pattern

Most Diptera, and all Brachycera, show some degree of fusion of neuromeres to form the TAG. We find a progressive tendency for higher degrees of VNC fusion in higher Brachycera. The basal pattern is 2 thoracic ganglia, (T1-T2) and (T3-A1), 5 unfused abdominal ganglia (A2-A6), and a fused TAG. In no case did we see T1 and T2 as separate ganglia or a separation of T2 and A1 in Brachycera. In most Brachycera T1-3 and A1 fuse into a

compound ganglion, although most noticeably the Asiloidea and Nemestrinoidea retain the separation between T2 and T3. Of the 2 empidoid families, the Empididae retain the primitive separation.

Identification of the variable pattern elements

The greatest degree of variation we see in the Diptera is the fusion of abdominal neuromeres into either the TAG or the thoracic synganglion, or both. Posteriorward fusion of neuromeres into the TAG occurs most extensively in the Cyclorrhapha, although it is present to a lesser extent in the Xylophagomorpha, Stratiomyomorpha, Tabanomorpha, Nemestrinoidea and Asiloidea. Anteriorward fusion (fusion of abdominal neuromeres into the thoracic synganglion) has occurred only in the Cyclorrhapha and Dolichopodidae, although in most cyclorrhaphan families examined the phenomenon is restricted to a fusion of A2 into the T1-3/A1 synganglion. Thus, the progression in the VNC toward a higher degree of fusion may be divided into six separate processes: (1) fusion of T1 and T2 in lower Diptera, (2) fusion of T3 and A1 in lower Diptera, (3) fusion of the T1-2 and T3/A1 ganglia in the Xylophagomorpha, Stratiomyomorpha, Tabanomorpha and Cyclorrhapha, (4) progressive increase in the number of neuromeres comprising the TAG in the Brachycera, (5) anteriorward fusion of abdominal neuromeres in Cyclorrhapha, and (6) the complete fusion of these anterior and posterior neuromeres into a synganglion. The progression of intermediate forms suggests that complete fusion of all neuromeres of the VNC into the synganglion is likely the end-point of both anteriorward and posteriorward fusion phenomena.

A novel morphological synapomorphy in the lower Brachycera

Current research on the phylogeny of the lower Brachycera using quantitative analyses of morphological characters provides weak evidence for a clade that contains the Xylophagomorpha, Stratiomyomorpha and Tabanomorpha (Yeates 2002). This clade is also supported if the homodactylus nature of the pretarsus in these infraorders is considered a synapomorphy (Stuckenbergh 2001). Our research presents another novel synapomorphy that is consistent with this grouping: the fusion of all thoracic ganglia and the 1st abdominal ganglion. The Rhagionidae (Tabanomorpha) we examined exhibited a narrowing between neuromeres T2 and T3 associated with a thoracic phragma. However, this was not distinct enough for us to consider it the plesiomorphic state. Perhaps the condition found in Rhagionidae represents an early stage in the fusion of these ganglia. This is consistent with paleontological evidence that rhagionids were amongst the first Brachycera to appear in the fossil record (Woodley 1989).

A cyclorrhaphan synapomorphy evident in our data is the fusion of all ganglia into at most 3 separate groupings, usually one thoracic and one abdominal ganglion, or a synganglion. The Syrphidae are the only cyclorrhaphan family with the A3 ganglion distinctly separate from the other abdominal ganglia. The Calyptratae all have a synganglion. However, this feature undergoes homoplasy according to our phylogeny, occurring also in the Dolichopodidae, Phoridae, and the closely related acalyptate families Ephydriidae and Drosophilidae. Another synapomorphy is present within the acalyptates: families from the Conopidae to Tephritidae share fusion of A2 into the T1-A1 neuromere. Increased sampling will test the veracity of all these hypotheses of synapomorphy. However, Fritz (2002) has recently confirmed our results in a different genus of Tephritidae.

VNC structure and body shape

We considered the notion that VNC fusion in Diptera is a function of body shape rather than a broad phylogenetic trend as we have proposed. Perhaps the trend towards more broad, short and compact body shapes in the Diptera can explain the observed neuromere pattern: those flies with more compact bodies having more compact and fused VNCs. We tested this hypothesis by comparing the VNCs of two sets of closely related (cofamilial) flies having very different body shapes, one member of each pair being exceptionally elongate and the other being short and broad-bodied. The comparisons were made within families encompassing a diversity of body shapes, the Bombyliidae and Syrphidae. We found that the family-specific pattern of the VNC is retained in both extremes of body shape. Although these observations do not completely refute the above hypothesis, they suggest that a reversal in VNC fusion level is not a common phenomenon. In fact, VNC fusion in the Diptera appears to evolve under Dollo's law of irreversibility (Gould 1970). Various levels of VNC fusion can evolve and have evolved independently, however.

Adult and larval VNC structure

Ventral nerve cord architecture in the larval stage has been subject to fewer studies than in the adult. However, Brandt (1879) depicted the change in fusion from larva through to the adult in 4 brachyceran families. It is obvious that ganglion structure of the larval VNC rarely matches that of the adult (Melzer et al. 1995). Melzer et al. (1995) compared the structure of the larval VNC in 17 families of Brachycera and found 4 levels of neuromere fusion. Complete fusion, with all neuromeres contracted into a single mass ("peg"), was only found in the Cyclorrhapha and Tabanidae, and a lesser degree of fusion ("5-limbed chain") was found in the Xylophago-

morpha, Asiloidea, and some basal tabanomorphs (Rhagionidae and Vermileonidae). Comparing their data with currently accepted lower brachyceran relationships (Fig. 1) indicates that the evolution of larval VNC ganglion structure has included some homoplasy. While the relevant comparisons within species have been made in only a few cases, it is highly likely that larval and adult VNC fusion is decoupled in ontogeny.

Studies of VNC formation and arrangement through metamorphosis may provide useful information on the various independent pathways and processes leading to the dipteran synganglion. For example, the fused thoracic synganglion of Phoridae or Dolichopodidae may come about through a different developmental mechanism than in the Muscoidea. Studies of taxa unavailable to us – e.g. the Vermileonidae, basal rhagionids such as *Austroleptis*, and Pantophthalmidae – will further refine the distribution of synapomorphies in the lower Brachycera.

Functional basis

Functional advantages of fusion that have been suggested include reduced neural conduction times, the elimination of relay interneurons, and increased availability of sensory input to arrays of neurons (Leise 1991). The observation that the thoracic neuromeres – the centers of locomotion – are most commonly fused in insects suggests that neuromere fusion enhances the coordination of complex behaviours requiring rapid, coordinated intersegmental sensori-motor activity. Boyan & Ball (1993) point out a correlation between fusion of body segments and fusion of neuromeres, raising the possibility that differences in neuromere fusion between groups are not necessarily functional. However, our results show that VNC fusion occurs readily in the absence of segmental fusion. It is conceivable that VNC architecture could be a consequence of evolutionary changes in non-neural tissues.

Acknowledgements

A grant from the Australian Research Council (98/ARCS156G) supported the bulk of this research. The Schlinger Foundation supported the surveys that provided most of the material. S. Schrader kindly translated German texts.

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Appendix

List of species examined (families in alphabetic order)

Family	Genus and species	Family	Genus and species
Acroceridae	<i>Mesophysa</i> sp.	Pipunculidae	<i>Cephalops</i> sp.
Asilidae	<i>Colepia</i> sp.		<i>Eudorylas</i> sp.
	<i>Daptolestes</i> sp.		<i>Metadorylas</i> sp.
	<i>Dolopus</i> sp.	Platystomatidae	<i>Duomyia</i> sp.
	<i>Laphria</i> sp.		<i>Euprosopia</i> sp.
	<i>Neoitamus</i> sp.		<i>Lamprogaster</i> sp.
Anisopodidae	<i>Ommatius</i> sp.		<i>Pogonortalis</i> sp.
	<i>Sylvicola</i> sp.		<i>Rivellia</i> sp.
Apioceridae	<i>Apiocera</i> sp.	Pyrgotidae	<i>Cardiacera</i> sp.
Bibionidae	<i>Dilophus</i> sp.	Rhagionidae	<i>Chrysopilus</i> sp.
	<i>Plecia</i> sp.	Sarcophagidae	<i>Sarcophaga</i> sp.
Bombyliidae	<i>Exoprosopa</i> sp.	Scenopinidae	<i>Metatrichia</i> sp.
	<i>Systropus doddi</i> Roberts	Sciaridae	<i>Sciara</i> sp.
Calliphoridae	<i>Calliphora</i> sp.	Stratiomyidae	<i>Acanthasargus</i> sp.
Ceratopogonidae	<i>Culicoides</i> sp.		<i>Actina</i> sp.
Chloropidae	Gen. et sp. indet.		<i>Exaireta</i> sp.
Culicidae	<i>Anopheles</i> sp.		<i>Hermetia</i> sp.
Conopidae	<i>Australoconops</i> sp.		<i>Inopus</i> sp.
	<i>Microconops</i> sp.		<i>Odontomyia</i> sp.
	<i>Neoconops</i> sp.	Syrphidae	<i>Allograpta</i> sp.
Dolichopodidae	<i>Amblypsilopus</i> sp.		<i>Ceriana</i> sp.
	<i>Chrysosoma</i> sp.		<i>Episyphus</i> sp.
	<i>Neurigona</i> sp.		<i>Eristalinus</i> sp.
Drosophilidae	<i>Drosophila</i> sp.		<i>Eumerus</i> sp.
Empididae	<i>Empidinae</i> sp.		<i>Melangyna</i> sp.
Ephydriidae	<i>Scatella</i> sp.		<i>Microdon</i> sp.
Heleomyzidae	<i>Diplogeomysa</i> sp.		<i>Psilotia</i> sp.
	<i>Tapeigaster</i> sp.	Tabanidae	<i>Xanthogramma</i> sp.
Lauxaniidae	<i>Sapromyza</i> sp.		<i>Cydistomyia</i> sp.
Micropezidae	<i>Cothornobata</i> sp.		<i>Dasybasis</i> sp.
	<i>Metopochetus</i> sp.		<i>Ectenopsis</i> sp.
	<i>Mimegralla</i> sp.	Tachinidae	<i>Scaptia</i> sp.
Mycetophilidae	<i>Mycetophilinae</i> sp.		<i>Prosena</i> sp.
Muscidae	<i>Musca</i> sp.	Tephritidae	<i>Rutilia</i> sp.
Mydidae	<i>Miltinus</i> sp.		<i>Bactrocera</i> sp.
Nemestrinidae	<i>Trichophthalma</i> sp.	Therevidae	<i>Agapophytus albobasalis</i> Mann
Neriidae	<i>Telostylinus</i> sp.		<i>Anabarhynchus</i> sp.
Pelecorhynchidae	<i>Pelecorhynchus</i> sp.		<i>Nanexila paradoxa</i> Winterton & Irwin
Phoridae	<i>Megaselia</i> sp.	Tipulidae	<i>Limoniiinae</i> sp.
			<i>Exeretonevra angustifrons</i> Hardy
		Xylophagidae	