Coming apart at the seams: morphological evidence for pregnathal head capsule borders in adult Tribolium castaneum

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Abstract Cephalization and seamless fusion of the anterior body segments during development obscure the segmental boundaries of the insect head. Most of the visible seams are thought to reflect cuticular infolding for structural reinforcement rather than a merger of cuticular plate borders. Incomplete fusions and other modifications of the adult head found in eight Tribolium mutations indicate that the frontal and gular sutures likely are true sutures that mark borders between adjacent cuticular plates, and suggest that the anterior facial shelf is a composite of three independent cuticular surfaces: ocular, antennal, and clypeo-labral. Additionally, midline splits of the clypeo-labrum and gula, and membranous lesions on the lateral head capsule reveal probable borders of adjacent cuticular plates where visible sutures are normally absent. The anterior lateral lesions seen in the Lucifer mutation mark a border between ocular and antennal plates and appear to identify part of the postfrontal sutures. While revealing or clarifying possible intersegmental borders between ocular, antennal, and clypeo-labral plates, the various modified or unfused surfaces of the head neither reveal an additional acronal plate nor support the view that the clypeo-labrum is segmentally associated with ocular cuticle.

Keywords Tribolium castaneum · Head development · Procephalon · Suture · Labrum · Acron

Introduction

For over 100 years, the perceived acron of the arthropod head has been shrinking as additional areas of the fused pregnathal head have been found to represent segments. The acron was originally proposed to be a non-segmental end cap anterior to the serially homologous segments (Heymons 1901) and included the eyes and clypeo-labrum. It was regarded as the equivalent of the annelid prostomium (Snodgrass 1928). Other authors (DuPorte 1963; Butt 1960) considered the antennae also to be part of the acron. After many years of debate (Rempel 1975), more recent molecular and histological techniques have established that the insect pregnathal head includes intercalary, antennal, and ocular segments (Schmidt-Ott and Technau 1992; Rogers and Kaufman 1997), or more conservatively, an ocular or ocular/protocerebral region (Posnien et al. 2010; Scholtz and Edgecombe 2006), but these studies have not confirmed the existence of an acron. Additional studies of head segmentation in annelids and onychophorans found no evidence of an acron, but instead support homology of the onychophoran antennal segment (Mayer and Koch 2005; Eriksson et al. 2010) and the annelid prostomial brain (Tomer et al. 2010) with the insect ocular (protocerebral) segment. The segmental origin of the labrum is still controversial, and this structure has been variously thought to represent part of the acron (Snodgrass 1960; Matsuda 1965), a preoccular segment (Sharov 1966; Schmidt-Ott and Technau 1992), fused appendages (Butt 1960) or endites (Haas et al. 2001a, b) of a labro-intercalary segment, a part of the ocular/protocerebral region (Scholtz and Edgecombe 2006), a part of the pre-segmental ocular region (Posnien et al. 2009), or part of the ocular segment (Steinmetz et al. 2010; Eriksson et al. 2010).

Much of the difficulty in identifying adult arthropod head segments, or an acron, morphologically stems from the
absence of visible sutures that unambiguously define segmental borders. In larval, nymphal, and adult heads, the borders of gnathal and pregnathal segments are often obscured by extensive and seamless fusion of cuticular plates in the head capsule. When present, the locations of many sutures can vary among species. There is also disagreement about which of the apparent sutures reveal true cuticular plate boundaries and which represent cuticular infoldings for structural reinforcement (Snodgrass 1960; Matsuda 1965).

Many of the cuticular seams found in the head capsule were long referred to as “sutures” (Snodgrass 1928), implying that they indicated genuine cuticular plate boundaries. By 1960, Snodgrass had reinterpreted the morphological evidence, and had concluded that most suture-like grooves in the head, including the epistomal (es), occipital (os), and postoccipital (pos) sutures (Fig. 1a, b), were actually just cuticular infoldings (sulci) that served as either structures for muscle attachment or for cuticular reinforcement, rather than true sutures marking cuticular plate fusions. Somewhat ambiguously, he still proposed that the postoccipital sulcus marked a segmental border, perhaps inferring that head capsule segmental borders are permanently and irreversibly fused and incapable of generating true sutures. In direct contrast, Matsuda (1965) concluded that these same three grooves were the only true head sutures (i.e. those marking cuticular plate fusions). Snodgrass (1960) did recognize at least one true head suture, namely the median postgenal cuticular plate fusions. He regarded the ecdysial cleavage lines (frontal suture, found on the ventral head of some prognathous species) as lines of weakened cuticle that served to facilitate the splitting and shedding of the old cuticle at the time of molt (ibid.). In the present work, we use the term “seam” to refer to any visible crease or groove, while reserving the term “suture” to refer to specific seams that may represent true cuticular plate boundaries. Eight mutant phenotypes are described here and the relevance of the altered phenotypes to insect pregnathal head segmentation is discussed.

Materials and methods

Mutant stocks and culture methods Beetles were reared at 30°C on whole-wheat flour containing 5% (w/w) brewer’s yeast. The following mutant stocks were used or referred to in this work: Antennagalea1 (Ag1); Antennagalea2,Stumpy (Ag2,Stm); Antennagalea3,Stumpy (Ag3,Stm); antennapedia (ap); Cephalothorax5,Stumpy (Cx5,Stm); Cephalothorax6 (Cx6); Clef gula (Cg); Eyeless (Ey); Lucifer (Lu); maxillopeda (mnp); Notched (nerg); microcephalic (mc); prothoraxless (ptlD2); Split gula (Sg); Transformed labrum (Tlr). See Table 1 for additional details on origins and properties of mutant strains.

Microscopy Freeze-killed specimens for light microscopy were prepared by rinsing in a 2.5% bleach solution to remove flour debris, dried on lint-free tissue paper, and placed in 70% ethanol, a lactic acid/ethanol solution, or a commercial eye gel in a shallow-well glass microscopic slide or a glass ten-well plate. A Leica MZFLIII StereoScope with a Planapo ×1.0 objective and an attached Nikon DXM1200F Digital camera were used for photography.
Photos or photo sections were captured using Nikon ACT-1 photo capturing software. Digital image sections were merged using Helicon Focus software. Figures were created using the GNU Image Manipulation Program (v. 2.6.3) and Open Office (v. 3.2.1) software.

**Results and discussion**

**The wild-type head capsule**

The head capsules of a generalized insect and of *Tribolium castaneum* are shown in Fig. 1a, b, c, and e, respectively. Viewed dorsally, many appendages of the smoothly contoured head of *T. castaneum* are partially hidden under the facial shelf, a composite of the anterior rim of the genal (Ge) and clypeal (Cl) cuticle (Fig. 1c, d). The dorsal surface of the eye (E) is bluntly pointed anteriorly, and laterally it is narrowed by the gena extending into the eye surface (Fig. 1d). Ventrally, the eye enlarges and extends medially to almost one third of the head width (Fig. 1e). While the labial palps (Lbp), which are nested between the maxillary palps (Mxp), are hidden in lateral view (Fig. 1d), all mouthparts and antennae (Ab) are clearly visible ventrally (Fig. 1e).

**Visible seams**

The head capsule of *T. castaneum* (Fig. 1c–e) normally bears only two readily visible pairs of seams, namely the frontal sutures (fs) at the anterior and the ventral gular sutures (gs). A very faint postoccipital suture is found at the posterior border of the head. Several seams which are clearly visible in some insect species are, in *T. castaneum*, seen as very shallow grooves (occipital and epistomal, the latter being barely, if at all, visible). Laterally (Fig. 1d), the occipital groove (og) curves around the posterior and ventral perimeter of the eyes and terminates near the maxillary cardo (Mxc) sockets (Fig. 1e).

When present, the epistomal suture (Figs. 1a–d and 2a) is an important feature of insect head morphology. It marks the

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**Table 1** Alleles described in this work

<table>
<thead>
<tr>
<th>Allele name</th>
<th>Origin</th>
<th>Description and gene</th>
<th>Locus</th>
<th>Penetrance</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antennagalea¹ (Ag¹)</td>
<td>G, Georgia-1</td>
<td>D, L Cx, Dfd</td>
<td>LG2, H</td>
<td>Good</td>
<td>Stuart et al. 1991</td>
</tr>
<tr>
<td>Antennagalea² (Ag²)</td>
<td>G, Stumpy</td>
<td>D, L Cx, ptl</td>
<td>LG2, H</td>
<td>Moderate</td>
<td>Beeman et al. 1996</td>
</tr>
<tr>
<td>Antennagalea³ (Ag³)</td>
<td>G, Stumpy</td>
<td>D, L mxp</td>
<td>LG2, H</td>
<td>Good</td>
<td>Beeman et al. 1996</td>
</tr>
<tr>
<td>antennapedia(ap)</td>
<td>S, Short antenna (Sa)</td>
<td>R, V ap</td>
<td>LG8</td>
<td>Good</td>
<td>Englert and Bell 1963</td>
</tr>
<tr>
<td>Cleft gula(Cg)</td>
<td>G, Ultrathorax¹</td>
<td>D, L?</td>
<td>LG9</td>
<td>Good⁴</td>
<td>This work</td>
</tr>
<tr>
<td>Cephalothorax⁵ (Cx⁵)</td>
<td>G, Stumpy/Stumpy</td>
<td>D, L Cx</td>
<td>LG2,H</td>
<td>Good</td>
<td>Beeman et al. 1996</td>
</tr>
<tr>
<td>Cephalothorax⁶ (Cx⁶)</td>
<td>G or EMS, Georgia-1</td>
<td>D, L Cx</td>
<td>LG2,H</td>
<td>Moderate</td>
<td>Beeman et al. 1989</td>
</tr>
<tr>
<td>Eyeless (Ey)</td>
<td>G, Georgia-1</td>
<td>D, L mc</td>
<td>LG2, H/LG5</td>
<td>Good</td>
<td>Beeman et al. 1996</td>
</tr>
<tr>
<td>Lucifer (Lu)</td>
<td>G, Socketless⁶/Stumpy</td>
<td>D, L ptl</td>
<td>LG2, H</td>
<td>Good</td>
<td>This work</td>
</tr>
<tr>
<td>Maxillopédia⁷ (mxp⁷)</td>
<td>G or EMS, Georgia-1</td>
<td>D, L mxp</td>
<td>LG2,H</td>
<td>Good</td>
<td>Beeman et al. 1989</td>
</tr>
<tr>
<td>microcephalic (mc)</td>
<td>S, Chicago wild type</td>
<td>R, V mc</td>
<td>LG5</td>
<td>Good</td>
<td>Sokoloff and Lasley 1961</td>
</tr>
<tr>
<td>prothoraxlessDominant2 (ptlD²)</td>
<td>G or EMS, Georgia-1</td>
<td>D, L ptl</td>
<td>LG2,H</td>
<td>Moderate</td>
<td>Beeman et al. 1989</td>
</tr>
<tr>
<td>Split gula (Sg)</td>
<td>S, Bar eye, sooty</td>
<td>D, L ptl</td>
<td>LG2, H</td>
<td>Good⁴</td>
<td>This work</td>
</tr>
<tr>
<td>Transformed labrum (Tlr)</td>
<td>EMS; Eyeless-lethal free</td>
<td>D, V ptl</td>
<td>LG2, H</td>
<td>Good⁴</td>
<td>This work</td>
</tr>
</tbody>
</table>

Cx Cephalothorax; D dominant; Dfd Deformed; EMS Ethylmethanesulfonate; G gamma irradiation; H homeotic complex; L Lethal; LG_/LG_ known translocation; mxp maxillopédia; ptl prothoraxless; R recessive; S spontaneous; V viable; “?” unknown

⁴ Based on unbalanced dominant stock expression (all others homozygous viable or balanced dominant lethal stocks)
border between the clypeus and frons, typically passing through the anterior tentorial pits (atp), with the anterior mandibular articulations at either end (Snodgrass 1928). In insects that lack a visible epistomal suture, the origin of the cibarial dilator muscles marks the clypeal surface, differentiating it from the frons (ibid). Several morphologists have identified different seams as the epistomal suture in various tenebrionids. El-Kifl (1953) identified an anteriorly arched seam under the facial shelf as the epistomal suture in *Tribolium confusum*. In contrast, Doyen (1966) maintained that the seam directly connecting the posterior ends of the frontal sutures was the epistomal (frontoclypeal) suture in *Tenebrio molitor*. El-Kifl labeled the equivalent shallow seam in *T. confusum* as the “anterior transverse groove”, but did not speculate on its significance. Doyen showed that the cibarial dilator muscles clearly originate on the dorsal head surface between the anterior rim of the facial shelf and the frontoclypeal suture, confirming that this surface is clypeal rather than frontal. According to Doyen’s interpretation, a portion of facial cuticle identified as frons by El-Kifl is actually clypeus, and the true frons has a more posterior location. Mutant phenotype interpretations presented here are based upon Doyen’s view of anterior head capsule morphology in tenebrionids.

Known unexpressed cuticular plate borders

Some universally seamless cuticle is known to be the fused medial surface of paired appendages. The clypeo-labrum (Cl, Lm, Fig. 1c), while usually formed from the fusion of two labral buds in the embryo (Scholtz and Edgecombe 2006), normally shows no intra-segmental medial seam as evidence of its dual origins, although its innervation does indicate a paired structure (Bocan et al. 2003). Similarly, on the ventral head of *Tribolium* and many other prognathous insects, the mentum (Mt), submentum (Smt), and gula (Gu) (Fig. 1e) show no medial seam, though they too are formed from the fusion of paired structures (Snodgrass 1960).

A visible suture sometimes reveals only a portion of a segmental boundary. The gular sutures, which are extensions of the postoccipital sutures (Fig. 1e; Snodgrass 1960), are found ventrally in many beetles and mark part of the border between adjacent maxillary and labial plates (Matsuda 1965). These sutures extend from the posterior head anteriorly to the posterior tentorial pits (ptp, Fig. 1d, e). There is no further, anteriorward extension of these sutures towards the bases of the maxillae, although such an extension would accurately mark what is considered to be the border between maxillary and labial cuticle (Figs. 1e and 2b, d). Thus, true interplate borders can be said to exist in otherwise featureless cuticle.

The location of the other sutures which mark adult head segment borders between ocular, antennal, and intercalary segments has been elusive. Histological techniques have demonstrated segmental boundaries in the heads of various arthropod embryos, including *Tribolium*, but have not provided insights into similar boundaries in the fused adult head. One complementary approach to embryo histology is the use of mutation-induced modifications of the adult head capsule to suggest possible segment borders. The eight mutants described below demonstrate a pattern of cuticular modifications which suggests several possible segmental borders.

Mutant phenotypes

Mutants described here represent probable homeotic mutations based on a variety of criteria: linkage to the homeotic complex, failure to complement known homeotic genes, a readily recognizable transformation and/or morphological similarity to known homeotic mutations (see Table 1 for details). While it is possible that features of a given mutant may be due, instead, to developmental defects, the speculations below assume that homeotic transformation is the most likely explanation for the observed morphological traits.
Future genetic and histological characterization of these mutants (work in progress) will help clarify the nature of the various cuticular modifications and further determine if they are homeotic transformations or developmental defects.

**Antennagalea**

*Antennagalea* (Ag) In addition to homeotically transforming, the antennal scape into a maxillary galea as described in Beeman et al. (1989) and Stuart et al. (1991), the Ag mutation also affects the facial shelf (Fig. 3b1, b2). The portion of the shelf covering the antennal base and lateral to the frontal sutures (antero-lateral facial shelf, Fig. 1c–e) can show a reduction in size, giving it a notched appearance and more fully revealing the antennal galea which projects dorsally from the basal scape segment. Occasionally, an additional reduction of the postero-lateral facial shelf near the eye is seen. The lateral eye remains emarginated, but with flat cuticle rather than raised cuticle, and the eye surface itself is not altered. The medial facial shelf between the frontal sutures is not affected. Antennae are held more ventrally than in wild-type (data not shown).

*Antennagalea 5, Stumpy (Ag 5, Stm)*  Ag 5, Stm also has a notched facial shelf (Beeman et al. 1996) (Fig. 3c1, c2) in addition to an occasional posteriorward homeotic transformation of the clypeo-labrum to a mandibular or maxillary identity (Haas et al. 2001a). The notch is the result of a shortening of the medial facial shelf between the frontal sutures (Fig. 1c). The facial shelf lateral to the sutures does not appear to be affected even though the antennae are transformed and, similarly to Ag, can show a subtle

**Fig. 3** Photographs (a1–k1) and generalized interpretive drawings (a2–k2) of wild-type and mutant phenotypes.  
*Colored areas show proposed segmental affiliation of affected surfaces. See Materials and Methods for abbreviations*
homeotic transformation of antennal scape to maxillary galea. The reduced medial shelf is a feature of \( Ag^5 \) rather than \( Stm \) since, in rare cases where \( Ag^5 \) segregates away from \( Stm \), the reduced facial shelf segregates with the non-\( Stm \) beetles. Antennae are held more ventrally than in wild type (data not shown). The appearance of galea-like projections on the antennal base and reduction of the medial facial shelf may represent reversions of these specialized structures, which normally lack endites (basal branches), to a more primitive state which lacks the derived basal cuticular out-folding (facial shelf) and expresses endites (galea and lacinia) as seen in the maxillary or mandibular appendages. \( Ag^5 \cdot Stm \) is a mxp mixed gain/loss-of-function mutation (Table 1). \( Stm \) (without \( Ag^5 \)) has also been shown to be a mxp mixed gain/loss-of-function mutation (data not shown).

Thus, it is possible that the facial shelf modification and occasional clypeo-labral transformation in \( Ag^5 \cdot Stm \) may be due either to an expanded mxp effect or to the influence of an additional gene, possibly Df\( \delta \), affecting the clypeo-labrum separately from the mxp-induced antennal modifications.

Cleft gula (Cg) In beetles with the strongest phenotype, the gular sutures on the ventral head are “split” from the neck to the maxillary cardo, showing unsclerotized cuticular membrane (Fig. 3i1, i2). The maxillary cardo socket is split medially and the pointed lateral corner is pulled away from the maxillary base. The absence of stiff, sclerotized cuticle at the split sutures allows a lateral spreading of the ventral head, slightly increasing the distance between the eyes and giving the wider head a “walleyed” appearance ventrally. The labial palps, which are normally located between the maxillary palps and not visible laterally (Fig. 1d, e), often shift posteriorly and ventrally, becoming visible in lateral view (not shown). A similar phenotype is found in the Cephalothorax\( ^6 \) (Cx\( ^6 \)) mutant (Beeman et al. 1989). As in Cx\( ^6 \), the split gular sutures suggest a partial transformation of the labial segment to the prothoracic segment which has ventrally oriented appendages and which does not have cuticular plates fused to, and nestled between, those of an adjacent segment.

Eyeless (Ey) Strongly expressed Ey beetles can be mostly or completely eyeless (Fig. 3d1, d2), with the normally eye-bearing portion of the head capsule reduced and laterally compressed. The lateral portions of the facial shelf are missing. Ventrally, the lateral corners of the maxillary cardo sockets are blunted compared to their more sharply pointed shape in wild-type (cslc, Fig. 1d, e). This blunting suggests that some portion of this surface may be associated with the ocular segment. \( Ey \), an apparent mutation in the microcephalic (mc) gene on LG5 and a translocation that maps to both LG2 and LG5, (Beeman et al. 1996), is likely a gene regulated by the Pax-6 gene eyeless (ey) located on LG4 (Yang et al. 2009). Phenotypes of Ey and Drosophila ey are similar in that no new structures appear that can be compared to structures on other segments (Halder et al. 1995). Instead, there is only a loss of structures. In contrast, a new structure (occasionally antenna-like) is sometimes found in the Tribolium mutant microcephalic (the variant referred to as microcephalic\(^{eyeless} \) (mc\(^{E} \)) (Sokoloff and Lasley 1961), suggesting that the ocular region is capable of homeotic transformation.

Lucifer (Lu) Two categories of novel features are found on the Lu head capsule (Figs. 3e1, e2, k1, k2 and 4a–g): (1) new cuticular structures and (2) modifications to the shape and texture of existing features. Most of these features, which show highly variable expression, are found on what are proposed to be the pregnathal portions of the head.

Dorsal horns (“H”, Fig. 4a, b, g): Horn-like structures of variable size are usually found on the dorsal head above the antennal sockets. The horns often have a single blunted or pointed tip, but occasionally bear a bi-toothed tip. When weakly expressed, the horns usually project antero-laterally, but when more strongly expressed, the tips are curved and can point antero-medially.

Setiferous pits (“SP”, Fig. 4a): When present, large setiferous pits are postero-medial to the horns, but lateral to the dorsal membranous lesions (DL) described below. Setae project antero-laterally from these pits. The pits appear sclerotized and no underlying membrane is obvious externally.

Ventral tubercles (“T”, Fig. 4b): These tubercles are protrusions of varying size, usually found at the lateral edge of the maxillary cardo socket, pointing postero-laterally. The larger cylindrical protrusions may have either a concave or convex tip. Smaller projections always have a convex tip. Regardless of their circumference, their length varies, with the distal tip of the longest approaching the lateral margin of the head. When the head is laterally reduced in strongly expressed Lu, the tubercles can project slightly beyond the sides of the narrowed head.

Dorsal membranous lesions (“DL”, Fig. 4a, c, d): Membranous lesions (areas of unsclerotized cuticle) vary in shape, size, and number but always occur on a discrete part of the dorsal head, medial to the eyes and to the elevated dorsal surface that bears the horns and large setiferous pits (see below) and anterior to the occipital groove. The anterior-most lesions are never found beyond the anterior border of the large setiferous pits.
when the pits are present. Although from one to four lesions of various sizes have been found on the dorsal head, most often a bilateral pair of rounded or elongated lesions or dents are present. Elongated lesions tend to diverge anteriorly (Fig. 4c), occasionally creating a “v” or “u”-shaped lesion if they merge at their posterior ends (data not shown). Sometimes one enlarged lesion is found in the middle of the dorsal head (Fig. 4d), but size, length and width of such merged lesions can vary (data not shown). In weaker phenotypes, lesions are often replaced by shallow dents at similar locations.

Anterior lateral membranous lesions (“ALL”, Fig. 4b, g): Additional single, small membranous lesions usually appear at the dorso-anterior edge of the eye. They are always found at the intersection of the eye and the postero-lateral edge of the elevated cuticle (see below) which often overhangs the eye. In Lu/+ they are usually small, but the size varies somewhat depending on the severity of the head capsule transformation. The lesions become dorso-ventrally elongated in very strong Lu/+ , Lu/Ey, or Lu,mc/mc genotypes (Fig. 4e, f).

Posterior lateral membranous lesions (“PLL”, Fig. 4b): A bilateral pair of very small, single lesions may be found posterior to the lateral eye, apparently on, or just anterior to, the occipital groove. When very small, they appear as sclerotized pits, but larger ones may show unsclerotized cuticle (membrane). They are always smaller than the anterior lateral membranous lesions.

Anterior notch on facial shelf: The anterior rim of the head capsule usually bears a notch at the frontal sutures due to a shortening of the antero-lateral facial shelf. When strongly expressed, the resulting notch can expose the two basal segments of the antennae, though the exposed segments may not be visible dorsally if large horns overhang the notch.

Head capsule shape: The dorsal cuticle is elevated posterior to the clypeus and between the eyes. Especially notable is the elevation of the head capsule surface that bears the horns and large setiferous pits. This raised cuticular plateau, or “elevated surface” (“ELS”, Fig. 4b) appears smoother than adjacent cuticle due to a lower density of the small pits that give the normal cuticle its characteristic stippled appearance. The textual discontinuity (“td”, Fig. 4a, g) helps define a somewhat oval surface that includes cuticle lateral to the frontal suture and extending in a gradual curve postero-laterally to the dorsal margin of the eye where it somewhat overhangs the eye. This surface appears to be continuous with the antero-lateral facial shelf overhanging the antennae, and thus may define the border of a modified antennal segment. Compared to
wild type, the length of the head is often slightly shortened. It is possible that either the elevating dorsal cuticle, a reduction in the cuticular surface due to the dorsal lesions, or both, may affect head length. Posterior to the eyes, the head often appears slightly narrowed laterally.

Eye reduction: The thickened or elevated cuticular surface bearing the horns (described above) expands into the area usually occupied by the dorsal eye (Fig. 4a, b, g). The ventral part of the eye may also be reduced in size, suggesting that the dorsal reduction may not be solely due to the expanded antennal cuticle.

Antennal bleaching and posture: Lu antennae often have a bleached or faded appearance which is more severe in the club than in the funicle, and there may be occasional fusion of funicle segments. Antennae may also be carried somewhat more ventrally than wild-type (data not shown).

Other species with this unique phenotype: Correlates to the Lu mutant have also been reported in adults of two other beetle species, namely the Horned gena mutation in T. confusum (Sokoloff et al. 1981) and the V-grube mutation in T. molitor (Ferwerda 1928), the latter of which also affected larvae. Both were dominant mutations associated with dorsal horn-like projections, one or more membranous patches, and ventral tubercles. Sokoloff attributed the dorsal lesions in T. confusum to the presence of the horns causing a partial fragmentation of the frons. He accurately predicted that a mutant similar to Horned gena would be eventually found in T. castaneum. Since the general mutant head pattern is similar in all three species, and all are dominant mutations, two known to be linked to the Hom-C, it is possible that they share a common genetic basis, perhaps involving homeotic transformations of the antennal and ocular segments. In Drosophila melanogaster, a Dfd gain-of-function mutant causes reduced eyes and “bulbous growths” ventral to the eyes (Chadwick et al. 1990), both traits similar to features found in the Lu, Hg, and V-grube mutations. Lu has been found to be a pil loss-of-function mutation (Table 1), but complements Dfd (data not shown), showing that it is not a Dfd loss-of-function. Thus, cuticular modifications seen in Lu are, at least in part, likely to be an effect of pil, perhaps due to a pil gain-of-function in the pregnathal region. Future studies (work in progress) will help determine if all modifications are due to pil influence, or whether other genes, perhaps a gain-of-function in Dfd, may also be involved.

MaxillopediaNotched Gena (mxpNG) Similar to Ag1 (see above), a shortening of the mid-lateral facial shelf creates a notch lateral to the frontal suture (Fig. 3f1, f2). Often the posterolateral shelf which projects into the eye surface is further reduced without affecting the size of the eye itself. The eye remains emarginate with flattened cuticle. In contrast to Ag1 and Ag5.Stm, the antennal scape does not bear a galea-like projection, making the nature of the phenotype less clear. mxpNG fails to complement maxillopedia (mxp) (Beeman et al. 1989) and thus, may be a simultaneous loss-of-function and gain-of-function in that gene. Ag1, which has a similar notch, is not a mxp loss-of-function (data not shown), but could be a mxp gain-of-function (in addition to a Dfd and Cx loss-of-function, Table 1). The assumption of a gnathal identity by the antennal segment may account for the notch in the facial shelf, a structure not present in gnathal segments. It is also possible that additional genes such as Dfd may be responsible for the notch in mxpNG. Future studies should help clarify which additional genes, if any, contribute to the phenotype.

Split gula (Sg) In strongly expressed Sg mutants, the gula, mentum, and submentum of the labial segment (Fig. 1e) are split at the midline, showing unsclerotized cuticular membrane (Fig. 3j1, j2), and the labial palps can be incompletely nested between the maxillary palps. The absence of sclerotized cuticle at the midline allows a lateral widening of the head similar to that seen in Cg, but usually weaker. The midline split appears to result from incomplete midline fusion of the labial appendage bases during adult head development. Sg also bears shallow dents on the dorsal pronotum, similar to prothoraxless (ptl), and non-lethally fails to complement pil (data not shown). Thus, Sg may be a simultaneous loss-of-function and gain-of-function in pil. A limited midline split (mentum only) is also found in the mutant prothoraxlessDominant2 (ptlD2) (Beeman et al. 1989) and is interpreted as an anteriorward transformation of the labial segment to a maxillary segmental identity.

Transformed labrum (Tlr) Weak transformations may include either a subtle midline desclerotization of the labrum or a deep medial cleft in the clypeus and/or labrum (Fig. 3g1, g2). The length of the longest clypeal clefts are similar to the length of the frontal sutures on the dorsal head. Stronger expression may produce an elongated clypeo-labrum with one or two mandible-like projections similar to those occasionally found in Ag5.Stm (Haas et al. 2001a), and like Ag5, Tlr appears to represent a homeotic transformation of clypeo-labrum to mandible. The gene responsible for this transformation is not yet known, but may be a gain-of-function in a gene controlling mandibular development, such as Dfd. In contrast to Ag5, no part of the facial shelf is shortened in Tlr, and Tlr antennae are normal. Individual beetles with similar
transformations of the clypeo-labrum have also been found sporadically in the following *T. castaneum* mutant lines: Lucifer (Lu); Antennagalea*4, Stumpy (Ag*4, Stm); and Cephalothorax*5, Stumpy (Cx*5, Stm).

Rationales for designating a seam as a true suture

A seam resulting from a cuticular infolding (sulcus) solely for the purpose of structural reinforcement or muscle attachment does not mark the border between cuticular plates, and thus is not a true suture. If the infolding is reduced to the extent that the seam is eliminated, a flattened, sclerotized surface would be expected, rather than an apparent split between plates.

In contrast, a true suture is a seam or groove that marks the border between two adjacent, “formerly distinct” cuticular plates (sclerites) (Chapman 1971). If one or both adjacent plates withdraw from their mutual border, an expanse of unsclerotized, unpigmented cuticle would be seen, creating the appearance of a “split” in the cuticle at that border. In other words, the two plates once again become “distinct”. Thus, we interpret the appearance of a split in either a seam or in a normally fused surface as evidence of a border between adjacent cuticular plates, i.e., a true suture.

A good example of an existing seam in Tribolium that is likely to be a true suture (according to the criteria above) is found in the antennapedia (*ap*) mutant (Englert and Bell 1963). The full range of possible suture expression can be seen by comparing the normal pleurosternal suture on the ventral metathorax of *T. castaneum* with that of *ap*. While named for its antenna-to-leg transformation, the *ap* mutation also causes disruption of this suture. Normally the pleurosternal suture is expressed as a clearly defined sclerotized groove (Fig. 5a). However, in *ap*, that groove can either become split and show an intervening region of membranous cuticle (Fig. 5c), or become fused (Fig. 5d), eliminating most or all traces of the groove. Sometimes all three conditions are found along the same suture, illustrating the great flexibility of suture expression. If this seam were only a cuticular infolding (sulcus), it would appear either as a seam or as a fused surface; never as a split revealing cuticular membrane. Thus, in addition to illustrating how a seam may split, it also demonstrates how an apparently fused surface can be the site of an interplate border and be capable of splitting. In other words, the absence of a seam does not prove the absence of a plate border at that location.

Implications of mutant phenotypes for head capsule morphology

**Facial shelf composition**

Some seams, such as the frontal sutures, which do not expose membranous cuticle upon retraction of one or the other of the bordering cuticular plates, may still be true sutures. In Tribolium, these sutures do not expand into membranous rifts in any mutant, but they often mark the boundary of independently affected cuticular domains. For instance, *Ag*5,Stm (Fig. 3c1, c2) shortens only the medial shelf, while *Ag*4 (Fig. 3b1, b2), *Lu* (Fig. 3e1, e2), and *mxp*NG (Fig. 3f1, f2) shorten only the lateral shelf and have no effect on the medial shelf. The sclerotized lateral margin of the unaffected shelf rim becomes slightly rounded anteriorly in the absence of an adjacent surface.

In contrast to *Ag*1, *Lu*, *mxp*NG and *Ag*5,Stm, all of which generate notches at the frontal sutures, *Ey* affects only the postero-lateral edge of the facial shelf, causing either a reduction or deletion of that region of the shelf and of the eye and adjacent cuticle (Fig. 3d1, d2). Thus, the eye and the postero-lateral edges of the shelf can be modified independently of the more anterior portions of the shelf, and the well-delineated anterior boundary of the *Ey* effect does not correspond to the frontal suture. Rather, this boundary lies distinctly to the posterior of the frontal suture, suggesting the existence of a seamless interplate border between the affected ocular cuticle and the adjacent unaffected antennal cuticle (Fig. 2a, b). These apparently independent head
capsule modifications found in the various homeotic transformations described here suggest the existence of three corresponding pregnal compartments, namely ocular, antennal, and clypeo-labral (Fig. 2c, d).

**Ocular segmental borders**

The apparent seamless interplate border of the facial shelf that is revealed by the Ey mutation may thus represent part of the ocular-antennal border (the postfrontal suture of the primitive head, Fig. 1a, b). In Lu, the anterior lateral lesion (ALL, Fig. 4b, e, f) may also define a part of this border. When Lu is very strongly expressed, or is combined with Ey (Lu/Ey) or microcephalic (Lu,mc/mc) (Fig. 4e, f), the postero-lateral edge of the facial shelf is often greatly reduced and the anterior lateral lesion is ventrally elongated. The most ventrally extended lesion surface seen thus far in Lu is found at, or just ventral to, the level of the antennal socket, thus separating the entire lateral margin of the elevated antennal cuticle from the more posterior ocular cuticle on a line that coincides with the expected position of the postfrontal suture (not expressed in wild-type Tribolium). The absence of exposed membrane along the lateral margin of the unaffected facial shelf rim in Ey, Lu, Ag1, mxpNG, and Ag5,Stm, in contrast to the membranous split found between ocular and antennal cuticle in Lu, suggests that the dynamics of suture expression in a cuticular out-folding, such as the facial shelf, differs from suture expression on a cuticular surface at the main body wall.

At the border of the posterior eye and the occiput, the posterior lateral lesions (PLL, Fig. 4b) may represent an incomplete fusion of segmental plates. According to Posnien et al. (2010), the occipital groove is the probable line of fusion between gnathal and pregnal segments (Fig. 2c, d), with the pregnal surface largely composed of ocular cuticle (Posnien and Bucher 2010). Thus, both the anterior and posterior lateral lesions appear to mark disrupted borders between the ocular segment and the adjacent antennal segment to the anterior, and the gnathal segments to the posterior.

**Ocular and labral transformations uncorrelated**

Though one or more of the facial shelf compartments may be altered in a given mutation, the combination of an ocular and clypeo-labral transformation without an accompanying antennal transformation has thus far not been found. If the labrum is part of the ocular/protocerebral region (Scholtz and Edgecombe 2006), this combination would be expected. Even in beetles with the strongest Ey expression, the clypeo-labrum remains unaltered, suggesting a separate segmental identity for this structure. Inversely, in even the most strongly expressed Tlr, only the clypeo-labrum is affected while the eye remains unchanged. It is possible that the ocular–labral combination may be found in mutations yet to be discovered, or that Ey or Tlr may have subtle, possibly internal, transformations of the clypeo-labrum or eye not yet detected. However, the pattern of mutant head capsule compartment transformations found thus far suggests separate segmental origins for the ocular and clypeo-labral regions.

**Antennal, ocular and frontal cuticle, and dorsal head composition**

Posnien and Bucher (2010) find that, in Tribolium development, antennal, intercalary, and mandibular segments appear to contribute little, if at all, to the composition of the dorsal head, and suggest that the vertex of the larval and adult heads consists largely of ocular cuticle. The Lu adult phenotype seems to support this view, and demonstrates the relative absence of antennal cuticle on the vertex. If the textural discontinuity (Fig. 4a, g) accurately defines the borders of basal cuticle encircling right and left antennae, these two antennal surfaces do not meet at midline at any point on the dorsal head. The antero-medial borders of the bases are separated by the clypeus and frons, and the postero-medial borders separated by the dorsal lesions. However, these observations do not exclude an antennal contribution to the vertex because the exact posterior border of the Tribolium frons is unclear. If it does not abut the lesioned area in Lu, there could be a small surface of antennal cuticle meeting at midline. This would be possible if the obvious basal antennal cuticle is surrounded by less well-defined antennal cuticle which extends its borders, allowing some small surface of both halves to meet at the midline at the posterior frontal border and thus contribute to vertex cuticle (Fig. 2c).

The horns on the raised antennal surface of Lu are located over the ventral antennal socket and project dorsally, similar to the orientation of the galea found on the antennal scape in the Ag1 mutant. The orientation of both of these structures differs from that of the maxillary endites (galea and lacinia), which are found medial to the telopodite (palp). This relative rotation may be similar to the labral rotation previously noted in the developing Tribolium embryo by Kimm and Prpic (2006). Thus, appendages of pregnal segments may assume a slightly different orientation compared to gnathal segments.

A number of dorsal Lu features could be interpreted as homeotic transformations. The Lu horns may represent the appearance of enlarged lacinia on the antennal segment. Similar to the maxillary lacinia, the horns occasionally can be bi-toothed. Also similar to the maxillary lacinia, the horns are found on cuticle which is basal to the galea (antennal galea found in Ag1). However, the comparatively
large size of the horns in comparison to the maxillary lacinia, suggests that they may represent an antennal segment transformation to mandibular identity (greatly enlarged lacinia) rather than maxillary identity. This would also be consistent with the deteriorated structure of the antenna found in some Lu adults (bleaching and fusion of segments, data not shown), since the antennae are telopodites, an appendage structure which the mandibular segment lacks (Machida 2000; Boyan et al. 2002). Thus, development of the antenna itself could be incomplete or compromised in Lu. The ocular segment may also be transformed to a mandibular identity. Since the mandibular segment does not contribute to dorsal head cuticle (Posnien and Bucher 2010), the dorsal lesions could represent incomplete development or significant reduction of dorsal ocular cuticle. Such a reduction could also cause the shorter head length characteristic of Lu. The genetic basis for such transformations in Lu remains unresolved. This mutant has been shown to be a ptl loss-of function, but whether the Lu cuticular modifications result solely from a possible gain-of function in this gene, or from the abnormal expression of other mutated genes, remains to be determined (work in progress).

Of particular significance is what the Lu dorsal lesion pattern does not demonstrate. It does not delimit an additional independent plate in this area that might constitute the acron. The lesions also do not extend from the region between the eyes anteriorward into the frontal and clypeolabral area, further suggesting that these latter surfaces are unassociated with ocular cuticle and thus are not part of a combined ocular/protocerebral region as suggested by Scholtz and Edgecombe (2006). Based solely on the cuticular modifications found in Lu, this observation does not immediately rule out the clypeo-labrum’s possible identity as an independent structure, nor the possibility that an internalized acron may exist without external cuticular expression.

Sutures and ventral segmental surfaces

The Cg mutation (Fig. 3i1, i2) extends the line of the intersegmental gular sutures anteriorward and clarifies the lateral boundaries of the ventral labium (Fig. 2b, d). Lateral to this extended gular suture lies maxillary cuticle, which includes the surface of the lateral and dorsal head between the occipital groove and the postoccipital suture at the neck rim (Figs. 1, 2).

It remains to be determined what portion of the surface comprising the lateral half of the cardo socket (Figs. 1e, 2d, white surface) is maxillary, and whether some small part of it may include mandibular and/or intercalary cuticle. Additionally, the Ey phenotype suggests that some part may even be ocular, and the Lu tubercles add the antennal segment to the list of possibilities since Lu affects both ocular and antennal segments. No adult or larval mutant phenotypes studied thus far in T. castaneum have demonstrated possible borders between maxillary and mandibular, or mandibular and intercalary cuticle.

An intra-segmental border within the mentum, submentum, and gula is revealed by the Sg mutant (Figs. 2b and 3j1, j2). The medially split surfaces of these three structures show that these normally fused surfaces are composed of a pair of labial appendage bases.

Segmental origin of Lu tubercles

Some novel features of the Lu head are difficult to interpret. The dorsal large setiferous pits (Fig. 4a) are, thus far, unexplained curiosities with no apparent homology to other head structures. The segmental origin of the ventral tubercles (Fig. 4b) at the lateral edge of the maxillary cardo socket is also unclear, but unlike the dorsal large setiferous pits, they are not located on a surface obviously associated with antennal cuticle. As noted above, the segmental affinity of the tubercle-bearing surface (lateral half of the maxillary cardo socket) is unclear. Thus, the tubercles may be simple cuticular overgrowths or, if instead, they represent a homeotic transformation, the morphology of the tubercles do not readily suggest which segmental structure they are emulating.

Until the segmental identity of the tubercle-bearing surface can be determined these questions will remain unanswered. Perhaps future studies of internal morphology and innervation will clarify the segmental identity of these structures and the surface that bears them. Promising new transgenic techniques (Evans et al. 2009) may also help demonstrate the segmental identity of these structures and surrounding surfaces.

Interspecies suture variability and segmental surfaces

Snodgrass (1960) used the multiplicity of ecdysial cleavage line (epicranial suture) locations on various species to build his argument that these seams (the frontal and postfrontal sutures) were too variable to be true sutures. However, our evidence based on mutant phenotypes suggests that the frontal and postfrontal sutures are indeed true sutures. This suggests that it is more genetically efficient to repurpose an existing structure rather than create an entirely new one. Thus, facial sutures can be expressed as lines of weakened, less sclerotized cuticle to facilitate molting.

When the various ecdysial (epicranial) lines are combined on a hypothetical primitive (hypognathous) head, a possible segmental pattern emerges (Fig. 1a, b). Epicranial sutures are found most often either medial to the antennae (frontal) or lateral to the antennae (postfrontal) (Matsuda 1965). Although it is rare for these two versions of this
suture to appear together (Snodgrass 1935), their occasional co-occurrence suggests that they are not alternative manifestations of a single suture. Frontal sutures, when present, define the lateral and dorsal borders of the frons (or frons and clypeus when these two structures are fused in some species). They terminate ventrally near the anterior articulations of the mandible and just lateral to the anterior tentorial pits in the hypognathous head (Fig. 1a) (Snodgrass 1928). The postfrontal sutures, together with their probable ventral extensions, the subocular sutures (sos), appear to define the lateral borders of the presumed antennal segment, which extends anteriorly to the frontal sutures (Fig. 1a, b).

Viewing these two versions of ecdysial cleavage lines as separate sutures helps explain a previously reported observation. Sharov (1966) observed that the medial ocellus, always found on the frontal sclerite (between frontal sutures), can be joined by the paired ocelli in Plecoptera, suggesting an apparent migration of the pair. This assumption of ocellar migration could better be explained by attributing the differences to the variability of suture expression among species. For instance, when the frontal suture is present, only the median ocellus is obviously located on the frons (Fig. 1a, b). When the frontal sutures are missing but the postfrontal sutures are present, the lateral ocelli appear to have migrated to the frons from a non-frontal location, an illusion based on the variability of suture expression.

Snodgrass (1960) also pointed out that the weakened cuticle of some ecdysial cleavage lines even tears into the surface of the eye as the lines split during molting. This demonstrated that they cannot be “true” sutures marking segmental boundaries, since sutures do not run through the eye. However, the shape of the head in some species might cause a cleavage line to tear beyond its terminus, producing an extension that does not conform to the original direction of the cleavage line. Thus, the multiple routes taken by the tearing of ecdysial cleavage lines at molting in different species may be determined both by which suture is being expressed (frontal or postfrontal) and by the dynamics of mechanical stresses on the topographies of various head capsule shapes.

Future prospects

Mutation-induced modifications of the Tribolium head capsule have provided important new insight into the nature of the segmental fusions that give rise to this structure during development. However, the fact that the fused pregnathous head of Tribolium is highly derived limits our ability to interpret such mutations. It might be even more instructive to examine analogous mutations in a more primitive, hypognathous insect such as a grasshopper. Once the full range of genes responsible for mutations such as Lu, Ag', and Tlr have been identified, perhaps it will be possible to induce mutations of their orthologs in other insects for comparison. Additionally, the variable pleurosternal suture expression found in ap (Fig. 5c, d) could prove to be a useful tool. Once the genetic control of this process is understood, it may become possible to selectively induce suture splits in the pregnathal head capsule in various insect species. Perhaps, as insights gained from promising new techniques further refine our understanding of the insect head, it is now realistic to expect that, well before the end of the current century, the “endless dispute” (Rempel 1975) of head capsule composition will be resolved and the existence and location of the elusive acron will either be demonstrated to everyone’s satisfaction, or the concept will finally be retired as an historic artifact.

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