RESEARCH ARTICLE

Conservation, Innovation, and Bias: Embryonic Segment Boundaries Position Posterior, but Not Anterior, Head Horns in Adult Beetles

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ABSTRACT

The integration of form and function of novel traits is a fundamental process during the developmental evolution of complex organisms, yet how novel traits and trait functions integrate into preexisting contexts remains poorly understood. Here, we explore the mechanisms by which the adult insect head has been able to integrate novel traits and features during its ontogeny, focusing on the cephalic horns of *Onthophagus* beetles. Specifically, using a microablation approach we investigate how different regions of the dorsal head of adult horned beetles relate to their larval and embryonic counterparts and test whether deeply conserved regional boundaries that establish the embryonic head might also facilitate or bias the positioning of cephalic horns along the dorsal adult head. We find that paired posterior horns—the most widespread horn type within the genus—are positioned along a border homologous to the embryonic clypeolabral (CL)–ocular boundary, and that this placement constitutes the ancestral form of horn positioning. In contrast, we observed that the phylogenetically much rarer anterior horns are positioned by larval head regions contained firmly within the CL segment and away from any major preexisting larval head landmarks or boundaries. Lastly, we describe the unexpected finding that ablations at medial head regions can result in ectopic outgrowths bearing terminal structures resembling the more anterior clypeal ridge. We discuss our results in the light of the developmental genetic mechanisms of head formation in holometabolous insects and the role of co-option in innovation and bias in developmental evolution. *J. Exp. Zool. (Mol. Dev. Evol.) 00B:1–9, 2016.* © 2016 Wiley Periodicals, Inc.

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INTRODUCTION

Virtually all multicellular organisms can be viewed as mosaics of discrete traits that originated at different time points along a species' evolutionary history (Raff, '96; Carroll et al., 2005; Grimaldi and Engel, 2005). Novel traits, thus, had to find ways to integrate alongside preexisting structures without compromising ancestral functions (Wagner, 2005). At the same time, novel traits commonly brought with them—or acquired along the way—new features that now characterize their biological significance in extant species (Moczek, 2008; Wagner, 2011). The integration of form and function of novel traits is thus a fundamental process that characterizes the developmental evolution

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of all complex organisms (Raff, '96; Wagner, 2014). However, how novel traits and trait functions integrate into preexisting contexts remains poorly understood.

A growing body of work proposes that a major route for the integration of novel traits within already established trait complexes is provided by the differential reuse of preexisting gene network components (Carroll et al., 2005; Gilbert, 2006; Wagner, 2011). Such reuse may involve the recruitment of pathways into novel spatial domains, as in the recruitment of *distal-less* during the developmental evolution of butterfly eye spots or beetle horns (Carroll et al., '94; Moczek and Rose, 2009), or the retention of already existing gene expression into novel temporal domains, such as the recruitment of abdominal *Hox* genes during the evolution of photic organs in adult fireflies (Stansbury and Moczek, 2014). Alternatively, already existing gene networks may simply acquire additional, novel target functions, allowing them to regulate both ancestral and derived features in the same location and developmental stage, as in the reuse of the ancestral wing venation patterning machinery in flies, which became repurposed to also position derived wing spots (Gompel et al., 2005). In all cases, preexisting gene networks have strong potential to both facilitate the origin of novel traits as well as bias their subsequent diversification in specific ways. Here, we seek to begin investigating the mechanisms that facilitate and potentially bias the integration and diversification of novel head structures in insects.

From the stalks of stalk-eyed flies and the weevil rostrum to the cephalic horns of dung beetles, the dorsal head of adult insects constitutes an evolutionary hotspot for innovation and diversification (Grimaldi and Engel, 2005). At the same time, the dorsal insect head represents a critical part of a highly conserved and ancient trait complex, the insect head capsule, in existence since the origin of primitive insects over 420 MYA (Grimaldi and Engel, 2005). While embryonic head formation is well studied across diverse taxa including insects, our understanding of how embryonic insect heads transform into their larval or adult counterparts remains poor, due in part to the highly derived and nonrepresentative mode of head formation found in *Drosophila*. Recent work in the red flour beetle *Tribolium castaneum*, however, has begun to establish a morphogenetic framework—the *bend and zipper model*—which presently provides the most complete model for how the embryonic head transforms into its integrated larval counterpart (Posnien et al., 2010; Posnien and Bucher, 2010). Among others, this model posits that the anteriormost region of the embryo undergoes a dorsal bending, followed by an anterior expansion of subterminal regions. These regions subsequently wrap around the previously terminal anterior region and then fuse, or "zip" together, in the process creating a prominent suture line between the clypeolabral and ocular segments (CL-OC boundary), recognizable as an upside down Y on the dorsal larval head (Figs. 1A and B). However, no model exists that further describes ontogenetic transformations of the larval to the corresponding pupal and adult dorsal head for any insect.

Here, we utilize another beetle system, horned beetles in the genus *Onthophagus*, to investigate the basic mechanisms that may be positioning and integrating head horns within the adult dorsal head. Beetle horns constitute an evolutionary novelty, usually used as weapons in male combat, and develop postembryonically by extensive cell proliferation of localized regions of the dorsal head epidermis during the late larval to pupal transition (Moczek, 2005). *Onthophagus* head horns differ widely in shape and size as a function of sex and species, though much less so in position (Emlen et al., 2005). Instead, the majority of cephalic horns are positioned singly or in pairs on the posterior dorsal head between the eyes, whereas only a few species evolved more anteriorly positioned horns. Intriguingly, while *Tribolium* and *Onthophagus* beetles possess highly divergent adult head morphologies, the corresponding *larval* heads appear very similar: both exhibit many of the same landmarks in similar locations, including a very prominent putative CL-OC boundary, which raises the possibility that embryonic-to-larval head transformations may be conserved in both taxa (Figs. 1B and C). Importantly, *Onthophagus*, but not *Tribolium*, contains within the CL region itself additional, though shallow, suture lines (indicated by thin lines in all figures), which while shared across all *Onthophagus* species examined thus far cannot be broadly homologized across beetles families and instead reflect superficial, *Onthophagus*-specific ecdysal sutures that aid during the shedding of the larval head capsule during the larval-to-pupal molt (Moczek et al., 2006).

In this study, we sought to identify which larval dorsal head regions contribute to horn formation in adult *Onthophagus* and test the specific hypothesis that ancestral patterning mechanisms that instruct the formation of the embryonic and larval dorsal head of beetles may have facilitated and potentially biased the integration and positioning of cephalic beetle horns. Specifically, using a microablation approach we establish correspondence between larval and adult head regions across three *Onthophagus* species with diverse horn placements and known phylogenetic relations (Fig. 1D). We find that the most widespread type of horns, paired posterior horns, are positioned along adult head regions corresponding to the embryonic CL-OC boundary, that this placement constitutes the ancestral form of horn positioning, and that the phylogenetically much rarer anterior horns are positioned firmly within the CL segment and away from any obvious preexisting larval head landmarks or boundaries. Moreover, we unexpectedly find that ablations at medial head regions can result in ectopic outgrowths bearing terminal structures resembling the anterior clypeal ridge. We discuss our results in the light of what is known about the developmental genetic mechanisms of head formation in holometabolous insects and the role of cooption in innovation and bias in developmental evolution.

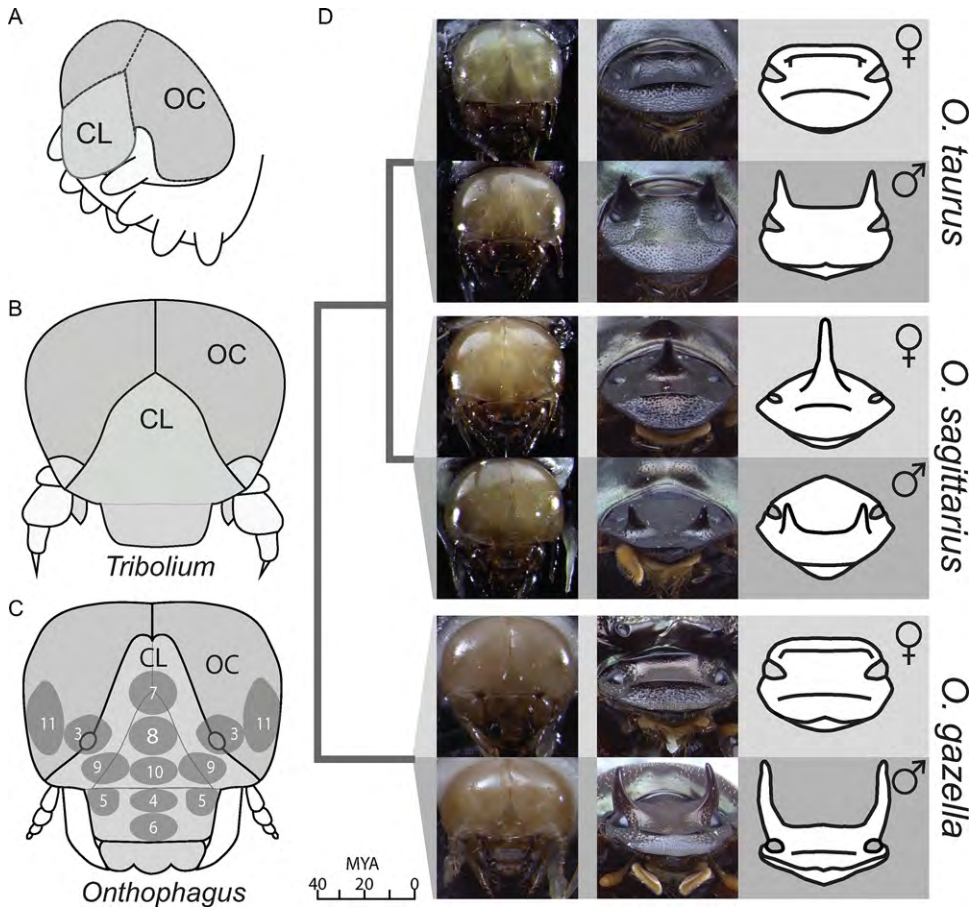

Figure 1. Diverse species in the genus *Onthophagus* possess very similar larval heads that develop into highly divergent adult morphologies. (A) Embryonic beetle head, showing the ocular (OC) and clypeolabral (CL) regions (after Posnien et al. (2010)). (B) Schematic representation of the larval head of the red flour beetle *Tribolium castaneum*, showing the ocular (OC) and clypeolabral (CL) regions. (C) Schematic representation of the larval head of a generic *Onthophagus* beetle, showing the ocular (OC) and clypeolabral (CL) regions. Medial and bilateral landmarks used for microablation are identified with numbers (see text). Note that in *Onthophagus*, but not *Tribolium*, the CL region itself contains additional, superficial suture lines (indicated by thin lines), which, however, cannot be broadly homologized across beetles families and instead appear to reflect superficial, *Onthophagus*-specific ecdysal sutures that aid during the shedding of the larval head capsule during the larval-to-pupal molt. (D) Larval and adult head morphologies of males and females of three species of *Onthophagus* beetles, and phylogenetic relationships among the three species, roughly proportional to time since divergence. Left column: Images of the larval heads of male and female *O. taurus* (top), *O. sagittarius* (center), and the more distantly related *O. gazella* (bottom). Center column: Corresponding adult heads photographed to indicate species-specific locations of head horns or ridges. Right column: Corresponding outline drawings used throughout the article to indicate key adult head features.

MATERIALS AND METHODS

Animal Husbandry

Onthophagus taurus individuals were collected near Bloomington, Indiana, whereas *O. sagittarius* and *O. gazella* were both collected from Oahu, Hawai'i. Laboratory colonies were maintained and bred as described previously (Moczek and Nagy, 2005). Briefly, larvae of each species were obtained by breeding sets of six females and three males in containers of packed moist soil with access to about 500 mL of fresh cow manure and allowing them to produce brood balls. Brood balls were collected after 8 days and animals transferred into 12-well plates after an additional 7–8 days as described in Shafiei et al. (2001). *Onthophagus taurus* larvae and pupae were kept in an incubator

at 24°C, whereas *O. gazella and O. sagittarius* larvae and pupae were kept in an incubator at 27°C. All species were maintained on a 16:8 hr light:dark cycle.

Ablation Experiments

A Hyfrecator 2000 electrosurgical unit (ConMed, Utica, NY) was fitted with a surgical tip (Epilation/Telangiectasia Needle Stealth ER coating 30 angle, 3/8″, 714-S; ConMed) to make precise ablations of head epidermis on the larval heads of all three species. A pilot study was conducted that tested three points along the suture line between the CL and OC segments to determine the range of intensities needed to generate ablations traceable to adulthood, replicability of ablation results, and the minimum area that needed to be treated to generate reliable results. During this initial pilot study, the duration of current application was varied from 1 to 3 sec depending on the species and the location of treatment, while current power ranged from 5 to 25 W. A power setting between 5 and 10 W yielded animals with clearly recognizable phenotypes yet without significantly increasing mortality. For the actual experiments, *O. taurus* larvae were treated for up to 3 sec depending on precise treatment location, whereas *O. sagittarius and O. gazella* were treated for only 1–2 sec as these species exhibited much greater treatment sensitivity. The power of the electrical current was kept between 6 and 10 W depending on the precise treatment location and species. All animals were treated during the last larval instar but well before animals entered the prepupal stage, that is, prior to any possible epidermal proliferation that normally enables horn formation. Lastly, each landmark was examined in on average five individuals per species and sex (see Table S1 in the Supporting Information for a detailed account of sample sizes).

Phenotype Scoring and Imaging

Images were taken through a Leica dissecting microscope (Leica, Buffalo Grove, IL) equipped with a digital color camera (Scion, Frederick, MD) using ImageJ (Abramoff et al., 2004). Phenotypes were scored based on the location and extent of damage in the adult dorsal head. Each animal was imaged once as a pupa and once as an adult at a 25× magnification. Extended focal depth composites for figures were generated by imaging representative individuals at ~20–30 focal planes and using the auto-align and auto-blend functions in Adobe Photoshop Creative Suite 6. Adult animals were stored in 70% ethanol and stored at 4°C for future imaging and analysis.

RESULTS

We utilized nine landmarks (named 3–11) reliably detectable on the larval head of three *Onthophagus* species to inflict localized damage to the dorsal head epithelium and to trace these injuries through metamorphosis to the pupal and adult stages (Fig. 1C). All landmarks yielded discrete and reproducible pupal and adult phenotypes, and bilateral landmarks yielded bilateral phenotypes. For example, injury to the lateral and medial clypeus in larvae resulted in corresponding defects in the formation of the lateral and frontomedial clypeus of adults (Figs. 2A and2 B). Similarly, damage to larval landmark 11, which appears in mature larvae as a faint horizontal line, reliably yielded near total ablation of the adult compound eye in all three species (Fig. 2C). Importantly, by examining both pupal and adult phenotypes we were able to identify possible compensatory or regenerative responses to our treatment during the pupal stage. In general, for almost all landmarks, adult phenotypes were slightly less severe than those seen in pupae, indicative of modest regenerative capacity during *Onthophagus* postembryonic development (Supporting Information Fig. 1). The only exceptions constitute landmark 11, where the adult phenotype was consistently considerably less severe than the corresponding pupal phenotype, and landmark 10, which results in a depression in the adult anterior ridge of females, but yields no pupal phenotype as ridges do not become visible until late pupal development. Taken together, we conclude that microablations can be used to reliably establish correspondence between larval and adult head regions for each species.

Adult Head Horns in *O. taurus* Are Positioned along a Conserved Segment Boundary

Using this approach, we next sought to test the hypothesis that segmental boundaries already established during embryogenesis are utilized to determine the placement of head horns during the formation of the adult head. To do so, we first focused on *O. taurus* and investigated a Y-shaped suture line prominent in larval heads, which demarcated the boundary between the embryonic CL and OC segments (CL-OC boundary). Ablation of larval landmark 3 along this suture consistently disrupted or eliminated left or right head horn formation depending on which side of the larval head was treated (Figs. 2D and 3A), supporting the hypothesis that in this species, adult head horns are positioned along the CL-OC boundary.

Different Larval Head Regions Contribute to Dorsal Head Horn Formation in Closely Related *Onthophagus* Species

We then repeated this approach in the closely related yet phenotypically divergent *O. sagittarius*. While both species share a common ancestor as recent as 10 MYA (Emlen et al., 2005), *O. sagittarius* females possess a single prominent medial head horn, whereas males develop paired horns on the anterior head (Fig. 1D). We sought to test whether the same or different larval head regions contribute to the differential placement of horns on the dorsal head of *O. sagittarius* adults. Ablation of the same larval landmark that disrupts horn development in *O. taurus* had no effect on horn development in either male or female *O. sagittarius*, suggesting different larval head regions enable horn growth in adult *O. sagittarius*. Instead, ablations of two distinct

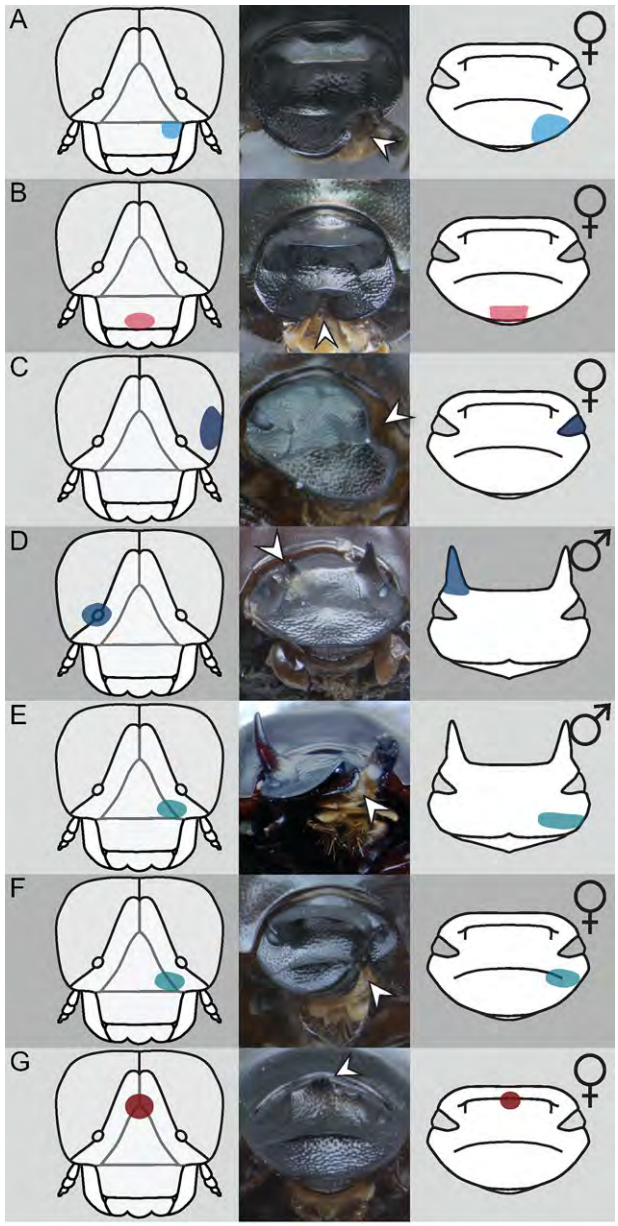

Figure 2. Results of microablations performed on the heads of *Onthophagus taurus* larvae. Left column: Schematic indicating larval head region targeted for microablation. Center and right columns: Images of the resulting adult beetle head phenotypes and corresponding outline drawings detailing the damaged or missing tissue in the lateral and medial clypeus (A and B), eye region (C), the posterior head (D and G), and the lateral dorsal head (E and F). Note that only microablations performed along the clypeolabral–ocular (CL–OC) suture line results in damage to or loss of the posterior horn in *O. taurus* males.

landmarks, 8 and 9, which are both positioned distinctly anterior to the CL-OC boundary, disrupted head horn formation in *O. sagittarius* females and males, respectively (Figs. 3B and C). Conversely, we found that ablations of these same regions in *O. taurus* did not affect head horn formation in *O. taurus* (Fig. 2E). Collectively, these results indicate that distinctly different larval head regions contribute to horn formation in *O. taurus* and *O. sagittarius*.

Horn Positioning in *O. taurus* Reflects the Ancestral Character State for the Genus

Next, we sought to determine which, if any, of the larval head regions thus far implicated by our results reflect the ancestral condition for horn formation in the genus. To do so, we repeated our approach in a third species, *O. gazella*. This species is more distantly related to both *O. taurus* and *O. sagittarius*, shares a common ancestor with both species approximately 40 MYA, and possesses broad, paired, posterior horns superficially similar in adult placement to those of *O. taurus* (Fig. 1D). After larval head microablations at landmarks 3, 8, and 9, only ablation at landmark 3 resulted in disruption of adult horn formation (Fig. 3D), suggesting that the use of the CL-OC boundary constitutes the ancestral location of head horn positioning in this genus. While the larval region that gives rise to the anterior head horn in *O. sagittarius* did not appear to contribute to the posterior head horn in *O. gazella*, its ablation did have an effect on the anterior female ridge in *O. gazella*, consistent with the notion that this region is responsible for both the anterior head horn in *O. sagittarius* males and the anterior ridge present in the females of all three species (Supporting Information Fig. 2).

Ablation of the Head Region that Gives Rise to Medial Head Horns in *O. sagittarius* Females Induces Ectopic Outgrowths in *O. taurus* and *O. gazella*

Recall that ablation of landmark 8 in *O. sagittarius* females disrupts the proper formation of the single medial head horn, resulting in many cases in a spectacular split-horn phenotype (Fig. 3B; Supporting Information Figs. 3A–C). Intriguingly, ablation of the same head region in *O. taurus* and *O. gazella* unexpectedly generated novel outgrowths on or slightly anterior to the posterior ridge. In both species, these ectopic outgrowths share characteristics with the anterior-most portion of the clypeus, including evenly spaced, dense bristles normally not found on this area of the dorsal head, but abundant on the ventral clypeal region (Supporting Information Figs 3D–I). This phenotype was observed both in *O. taurus* males and *O. gazella* females. In contrast, ablations at landmark 8 and the slightly posterior landmark 7 in *O. taurus* females resulted in abnormalities in the posterior ridge, but no clypeus-like outgrowths (Fig. 2G).

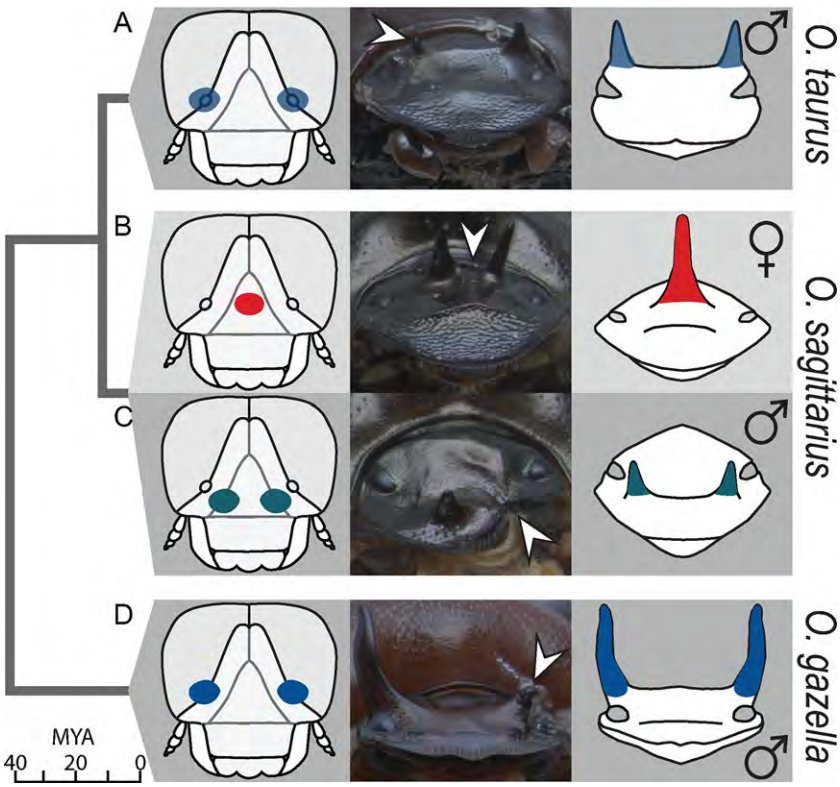

Figure 3. Comparisons of larval head regions that give rise to dorsal head horn formation in three species of *Onthophagus* beetles. Left and right columns: Outline drawings of larval and adult dorsal heads indicating location of microablations and the corresponding adult head region affected by this ablation. Center column: Images of representative adults illustrating (A) horn reduction phenotype in male *O. taurus*, (B) split horn phenotype in female *O. sagittarius*, and horn deletions in (C) male *O. sagittarius* and (D) male *O. gazella*. Note that despite being more distantly related to *O. taurus*, *O. gazella* males utilize the same larval head region to generate head horns, whereas more closely related *O. sagittarius* males and females do not.

DISCUSSION

In this study, we used a microablation technique to first establish correspondence between larval and adult head regions in three species of *Onthophagus* beetles, and then explored the relative contributions of different head regions to horn formation and positioning. We find that paired posterior horns—the most widespread horn type within the genus—are positioned along a boundary homologous to the embryonic CL–OC boundary, and that this placement constitutes the ancestral form of horn positioning. In contrast, we observed that the phylogenetically much rarer anterior horns are positioned by larval head regions contained firmly within the CL segment and away from any major preexisting larval head landmarks or boundaries. Lastly, we find that ablations at medial head regions induce ectopic outgrowths with striking morphological similarities to the anterior clypeal ridge. In the following, we discuss the most important implications of our findings.

Microablation as a Tool to Study Postembryonic Development in *Onthophagus*: Opportunities and Limitations

Our results demonstrate that simple, traditional microablations using a directed voltaic arc can be used across *Onthophagus* species to obtain basic fate maps that relate the hemispheroidal larval head to the mostly dorsoventrally flattened adult head (Fig. 4). As discussed in more detail below, such information is useful in order to homologize larval and adult head regions and identify larval head regions responsible for the postembryonic formation of adult traits such as horns. However, at least two important shortcomings remain. First, ablations, by definition, destroy cells, thus preventing these cells from proliferating, moving, or acting as a signaling center for neighboring cells, making it difficult to deduce the precise developmental mechanisms underlying specific ablation phenotypes. Second, in holometabolous insects adult phenotypes emerge as the product of developmental processes acting both during late larval

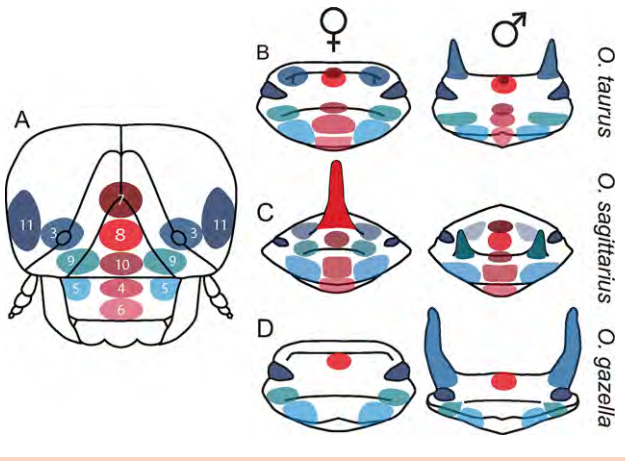

Figure 4. Fate maps relating larval head regions to their corresponding locations in the adult of males and females of three *Onthophagus* species. (A) Outline drawing of the larval head indicating locations of microablations. Center and right columns: Adult fate maps indicating corresponding ablation phenotypes in *O. taurus* (B), *O. sagittarius* (C), and *O. gazella* (D). Females are shown on the left, and males are shown on the right.

(prepupal) metamorphosis as well as the pupal stage, creating the possibility that later-acting developmental processes, for example, those acting during the pupal stage, may compensate for or otherwise obscure ablation phenotypes. Furthermore, even though we saw no obvious evidence for cell migration, we cannot discard that some growth compensation may be due to this process. Distinguishing local compensatory cell proliferation from cell migration would require the use of a tracer dye; however, this approach is technically impractical in *Onthophagus* as there is no technique available at present to dye regions of the head epidermis without lethally piercing the hard cuticle of the head capsule.

Despite these caveats, we generally observed strong correspondence between pupal and adult ablation phenotypes. For almost all landmarks, adult phenotypes were slightly less severe than those seen in pupae, suggestive of overall modest regenerative capacity during *Onthophagus* postembryonic development. The only two exceptions were landmark 11, which resulted in more noticeable regeneration of the lateral head, and landmark 10 whose adult phenotype becomes visible only in adults. Combined, this suggests that microablations can indeed be used to reliably establish correspondence between larval and adult head region in *Onthophagus* beetles.

Facilitation and Bias in the Origin and Diversification of Horn Positions

We found that paired posterior horns in *O. taurus* and *O. gazella*, two relatively distantly related species, derive from larval head regions positioned along a boundary that corresponds to the embryonic segment boundary between the CL and OC segments. Since both species share a common ancestor approximately as old as 40 MYA, shortly after the origin of the genus, these findings suggest that use of the CL-OC boundary to position head horns likely originated near the base of the *Onthophagus* radiation. Moreover, our results raise the possibility that part of the same developmental mechanisms that establish the CL-OC boundary during embryogenesis may have become repurposed to also instruct and position horn formation during late postembryonic development. If correct, such reuse may have facilitated the initial positioning of horns during early *Onthophagus* evolution, but may also have biased subsequent diversification of horn positions, given the disproportionate abundance of species with paired posterior horns across all regions of the clade (Emlen et al., 2005).

However, we also observed that not all adult horn positions derive from the same larval head regions. Specifically, we found that in *O. sagittarius* both the paired anterior horns found in males and the single medial horn found in females derive from larval head regions contained within the clypeolabral segment. These results raise the possibility that medial and anterior head horns as seen in *O. sagittarius* are not positioned relative to preexisting embryonic or larval segment or sclerite boundaries. If so, this raises the possibility that anterior/medial horns are positioned using a different, more recently derived positioning mechanism, possibly explaining why head horns in medial and anterior head regions are seen only infrequently across the *Onthophagus* phylogeny.

Ablation of Medial Head Regions Induces Ectopic Medial Outgrowths in *O. taurus* and *O. gazella*

While microablations at most larval dorsal head regions resulted in a subsequent loss of pupal and adult tissue, we unexpectedly found that ablation of the medial head region (landmark 8) instead reliably induced outgrowths in *O. taurus* males and *O. gazella* females. A possible explanation may be the existence of a signaling center located in that region that during normal development inhibits outgrowth formation, but becomes disrupted after ablation. Alternatively, or in addition, if regenerative processes are triggered upon ablation, it is possible that following the removal of tissues with medial identity, wound closure by recruitment of neighboring cell populations results in a positional mismatch, a phenomenon that is known to cause regeneration of ectopic structures in planarians and salamanders (Kato et al., '99; Gilbert, 2006). Intriguingly, a combination of these hypotheses may also explain a second, surprising finding, namely that the distal portion of the ectopic outgrowths induced after treatment of landmark 8 bears a striking resemblance to the normally more anterior clypeal ridge, including evenly spaced, dense bristles normally not found on this area of the dorsal head, but abundant on the ventral clypeal region. One possible explanation for this pattern is that, following ablation, more

anterior cell populations, which had already acquired clypeal identity, were recruited into the now disrupted signaling center, generating an ectopic outgrowth with clypeus-like features. However, none of these hypotheses explain the puzzling observation that ectopic outgrowths were never induced in female *O. taurus* or *O. sagittarius*. Regardless, even though clearly speculative at this point, these results raise the possibility that microablations in *Onthophagus* beetles may also be used to cautiously explore the dynamics of postembryonic developmental processes during insect metamorphosis, including possibly wound healing and regeneration.

From Fate Maps to Candidate Developmental Mechanisms for Dorsal Adult Head Patterning and Horn Positioning in *Onthophagus*

In this work, we present the first regional fate map relating larval and adult head morphologies across *Onthophagus* species, identify larval head regions critical to the positioning of adult head horns, and document that the nearly indistinguishable larval heads of different species are capable of generating diverse adult morphologies. Given the topological similarities between the larvae of *Onthophagus* and those of the red flour beetle *T. castaneum*, this map now allows us to tentatively connect adult head regions to their corresponding precursor tissue in the embryonic head. Given the growing understanding of the gene network patterning the embryonic *Tribolium* head (Li et al., '96; Posnien et al., 2010, 2011; Mahato et al., 2014), we are now in a position to investigate which members of these networks execute conserved roles during the patterning of the postembryonic head, which network interactions have been preserved or altered, and which genes if any may have been recruited to fulfill the new roles necessary to develop, position, and integrate novel structures such as cephalic horns.

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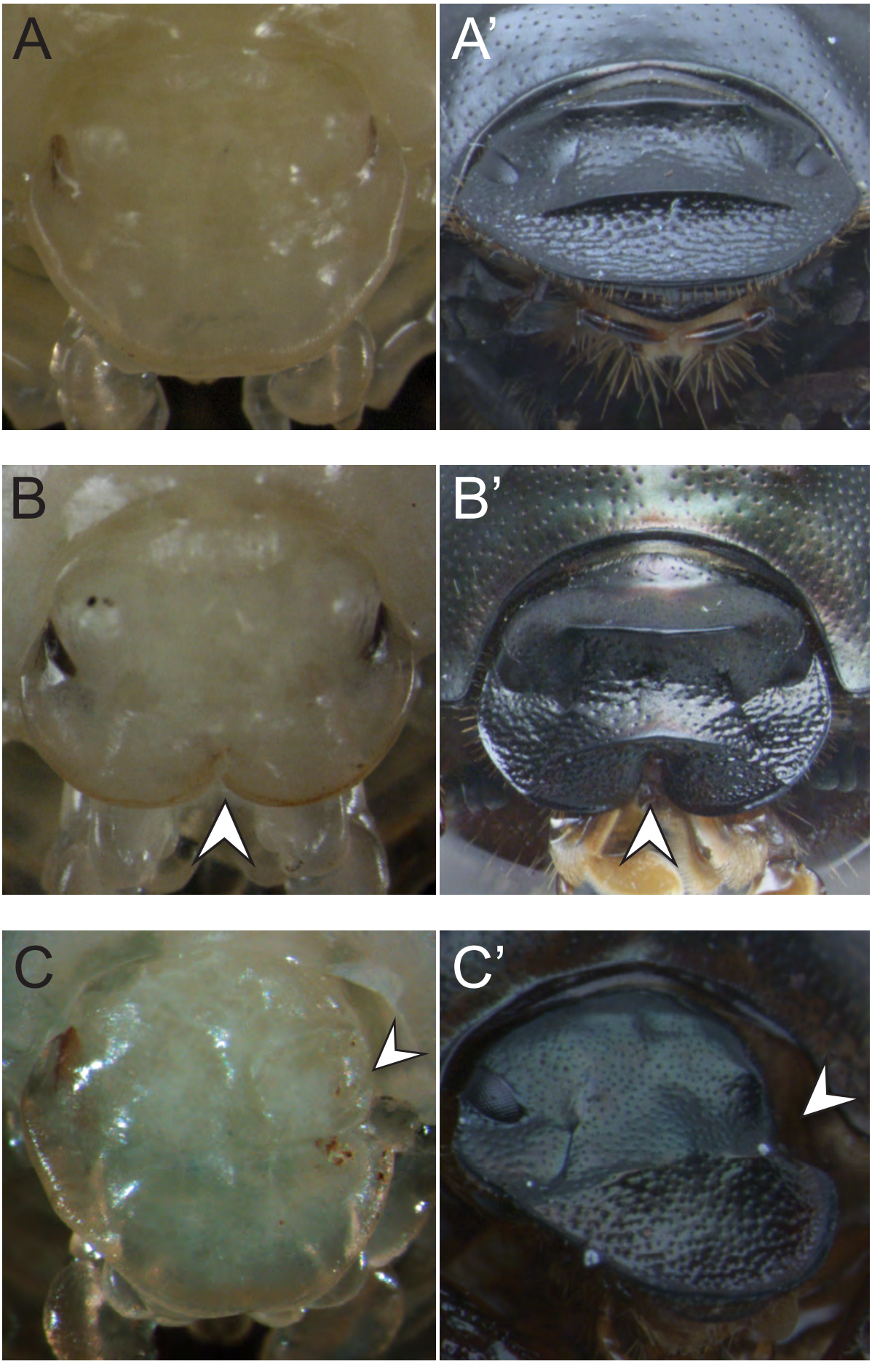
A
A’
B
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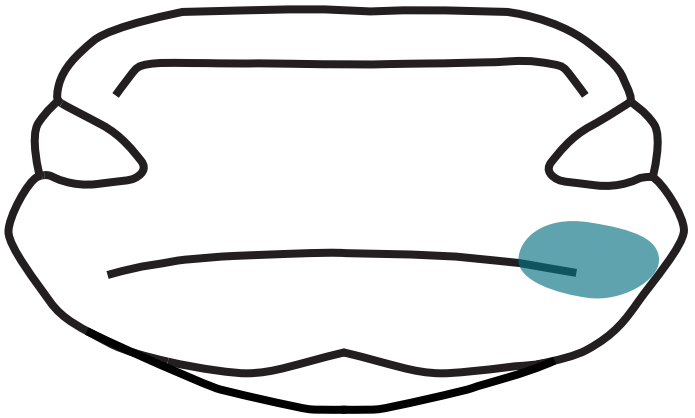
A

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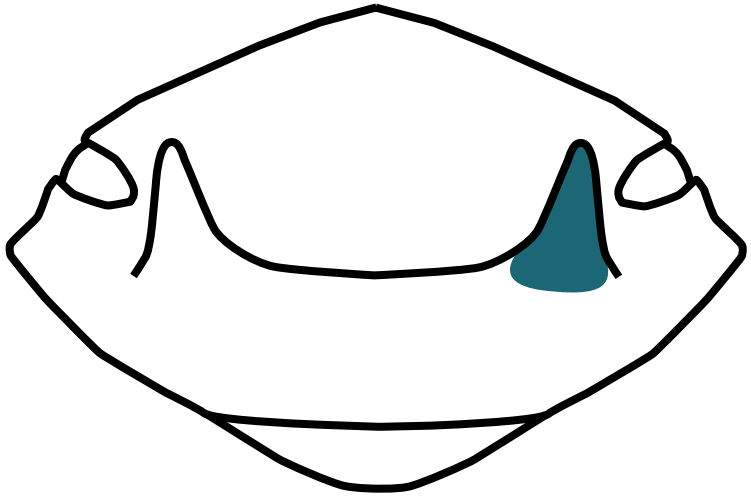

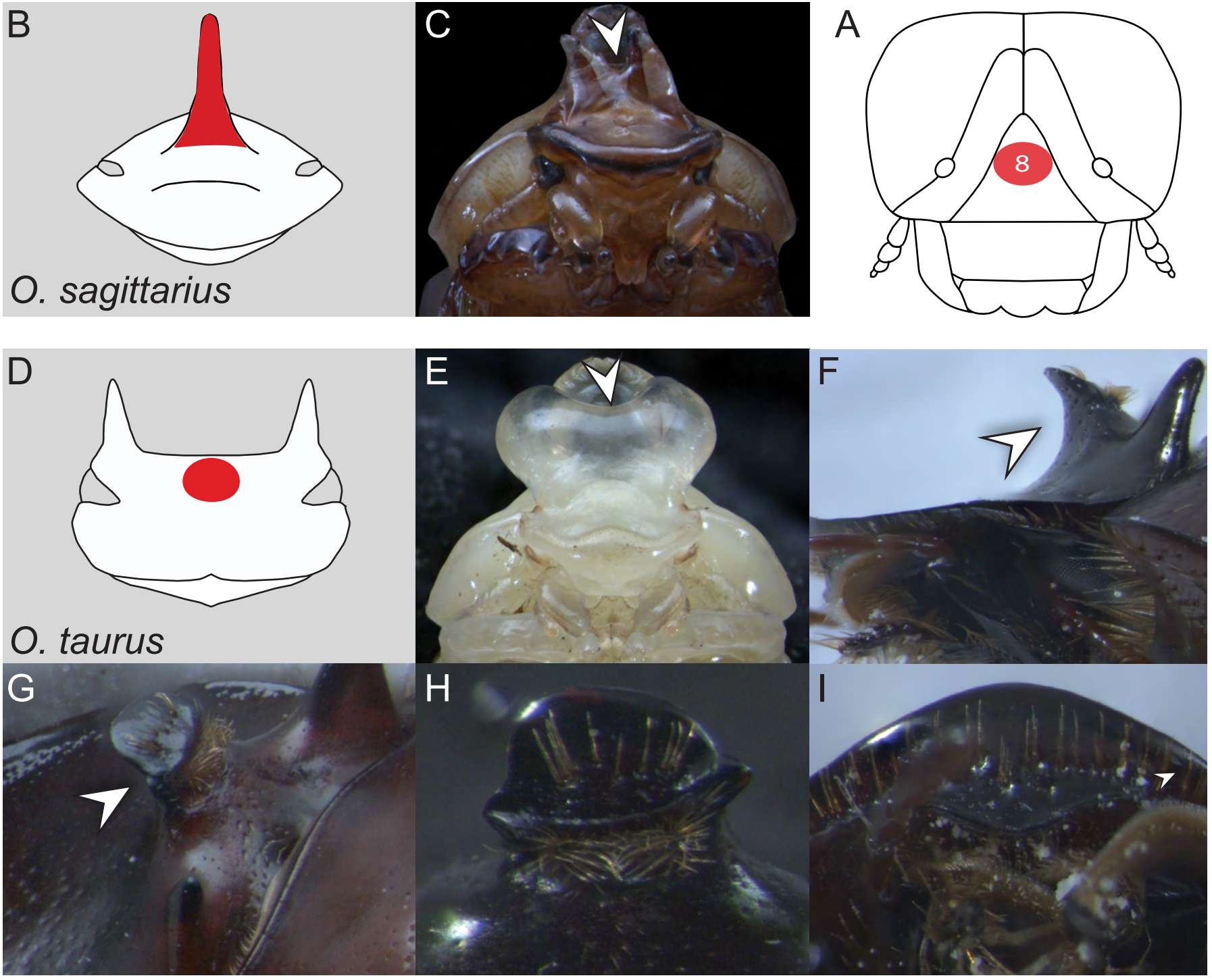
B
C
A
8
O. sagittarius
D
E
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O. taurus
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Supplementary Tables

Supplementary Table 1: Number of individuals showing pupal and adult phenotypes after ablation at each location. Adults are separated by sex (M: male / F: female).

	O. taurus		***O. sagittarius***		***O. gazella***	
Location	**Pupa**	**Adult (M/F)**	**Pupa**	**Adult (M/F)**	**Pupa**	**Adult (M/F)**
3	**14**	**9/2**	**7**	**3/2**	**15**	**7/2**
4	**30**	**12/4**	**6**	**3**	**-**	**-**
5	**7**	**1/1**	**3**	**2**	**-**	**-**
6	**3**	**2/1**	**-**	**-**	**-**	**-**
7	**5**	**2/2**	**3**	**1/1**	**-**	**-**
8	**11**	**7/3**	**23**	**1/16**	**8**	**1/1**
9	**10**	**3/6**	**25**	**19/2**	**10**	**7/0**
10	**3**	**1/2**	**5**	**2/2**	**-**	**-**
11	**-**	**-**	**27**	**9/3**	**3**	**1/2**