DIVERSITY IN THE WEAPONS OF SEXUAL SELECTION: HORN EVOLUTION IN THE BEETLE GENUS *ONTHOPHAGUS* (COLEOPTERA: SCARABAEIDAE)

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Abstract.—Both ornaments and weapons of sexual selection frequently exhibit prolific interspecific diversity of form. Yet, most studies of this diversity have focused on ornaments involved with female mate choice, rather than on the weapons of male competition. With few exceptions, the mechanisms of divergence in weapon morphology remain largely unexplored. Here, we characterize the evolutionary radiation of one type of weapon: beetle horns. We use partial sequences from four nuclear and three mitochondrial genes to develop a phylogenetic hypothesis for a worldwide sample of 48 species from the dung beetle genus *Onthophagus* (Coleoptera: Scarabaeidae). We then use these data to test for multiple evolutionary origins of horns and to characterize the evolutionary radiation of horns. Although our limited sampling of one of the world's most species-rich genera almost certainly underestimates the number of evolutionary events, our phylogeny reveals prolific evolutionary lability of these exaggerated sexually selected weapons (more than 25 separate gains and losses of five different horn types). We discuss these results in the context of the natural history of these beetles and explore ways that sexual selection and ecology may have interacted to generate this extraordinary diversity of weapon morphology.

Key words.—Adaptive radiation, beetle horns, character divergence, male competition, *Onthophagus*, phylogeny, sexual selection, weapons.

The history of life has been accentuated by several spectacular evolutionary radiations (Darwin 1871; Simpson 1949; Rensch 1959), and character divergence within these radiations frequently involves the exaggerated structures of sexual selection (Darwin 1871; West Eberhard 1983; Andersson 1994). The Hawaiian radiation of drosophilid flies, for example, has resulted in more than 900 extant species that differ most conspicuously in the wing patterns and courtship displays of males (Carson 1979; Templeton 1979; Kaneshiro and Boake 1987; Hoy et al. 1988; Kaneshiro 1988; Kambysellis et al. 1995). Similarly, radiations of birds-of-paradise, pheasants, and African Great Lake cichlids all involve the ornaments and displays of males (Beebe 1953; Gilliard 1969; Dominey 1984; Diamond 1986; Turner 1994; Deutsch 1997; Prum 1997; Frith and Beehler 1998; van Oppen et al. 1998). In fact, for a great many animals, the exaggerated structures of sexual selection display an evolutionary lability far surpassing that of other, not sexually selected structures (Darwin 1871; Geist 1966, 1978; Gilliard 1969; West-Eberhard 1983; Andersson 1994).

Diversification of the ornaments and displays of mate choice is understandable—even expected. Numerous studies suggest that female choice can drive rapid divergence among populations (Lande 1981; West Eberhard 1983; Kaneshiro and Boake 1987; Endler and Houde 1995; van Oppen et al. 1998; Boake 2000; Uy and Borgia 2000; Boughman 2001; Masta and Maddison 2002; Mendelson 2003; Coleman et al. 2004). Founder events, genetic drift, and minor geographic differences in natural or sexual selection all may alter the preference behaviors of females and generate divergent trajectories of male ornament evolution (Fisher 1930; Lande 1981, 1982; West-Eberhard 1983; Wu 1985; Lande and Kirkpatrick 1988; Schluter and Price 1993; Pomiankowski and Iwasa 1998; van Doorn et al. 1998; Higashi et al. 1999; Day 2000; Takimoto et al. 2000).

Weapons of male competition also have undergone conspicuous evolutionary radiations (e.g., antlers and horns of ungulates: Colbert 1955; Geist 1966, 1978; Vrba 1984; Kitchener 1985; Lincoln 1994; Vrba et al. 1994; Lundrigan 1996; flies: Dodson 2000; and ceratopsid dinosaurs: Lull 1933; Colbert 1965; claws of amphipods and isopods: Hurley and Jansen 1977; Shuster and Wade 2003; and tusks of frogs: Shine 1979; Emerson and Voris 1992; Emerson 1994, 2000), though *why* these have diverged in form is far less clear. Because weapons are not typically used as targets of active female mate choice (fiddler crab claws being a possible exception; Oliveira and Custodio 1998), links between weapon morphology and either assortative mating or population divergence are less obvious. It has even been suggested that male competition generates stabilizing selection on weapon morphology, a process that would prevent divergence in weapon form (Andersson 1994). Perhaps for these reasons, studies explicitly linking male competition with weapon diversification are almost nonexistent (see Geist 1966, 1978; West Eberhard 1983; Emerson 1994). We simply do not understand the evolutionary radiation of weapons with the same clarity that we do ornaments or displays.

Comparative studies are needed to characterize diversity in the weapons of sexual selection and to begin to explore why these structures may have diverged in form. Here we explore the evolutionary radiation of one type of male weapon: beetle horns.

A multitude of beetle species bear some form of enlarged horn in males. Isolated representatives of the Staphylinidae (e.g., *Bledius* sp., Arrow 1951; *Leistotrophus versicolor*, Forsyth and Alcock 1990; *Oxyporus* spp., Hanley 2001); Cerambycidae (e.g., *Macrodontia cervicornis*, Arrow 1951), Curculionidae (e.g., *Parisoschoenus expositus*, Eberhard and Garcia 2000), Tenebrionidae (e.g., *Bolitotherus cornutus*, Pace 1967; Brown and Bartalon 1986; Conner 1988), and

Chrysomelidae (e.g., *Doryphora* sp., Eberhard 1981) all produce either an enlarged form of mandibles or an enlarged cuticular projection of the head or thorax in males. However, the vast majority of the horned beetles cluster within the superfamily of chafers: the Lamellicornia. Thousands of species within this group, and in particular within the families Dynastinae, Lucanidae, Cetoniidae, and Scarabaeidae, are characterized by elaborate—even extravagant—cuticular weapons of males (Darwin 1871; Paulian 1935; Arrow 1951; Eberhard 1979, 1980; Otte and Stayman 1979; Enrodi 1985). Horn expression in these taxa is almost exclusively confined to males, and in every species studied to date, horns function as weapons in male competition over reproductive access to females and are not used as targets for any form of female choice (e.g., Eberhard 1978, 1982, 1987; Palmer 1978; Goldsmith 1987; Siva-Jothy 1987; Conner 1988; Otronen 1988; Rasmussen 1994; Emlen 1997; Moczek and Emlen 2000; J. Marangelo, pers. obs.).

In this study we focus on one genus of the family Scarabaeidae, *Onthophagus* Latreille (Coleoptera: Scarabaeidae: Coprinae: Onthophagini), and illustrate the evolutionary radiation of horns. With more than 2000 species already described, *Onthophagus* is the largest genus of beetles, and one of the most species-rich genera of life on Earth (Howden and Cartwright 1963; Matthews 1972; Howden and Gill 1993). *Onthophagus* is considered by taxonomists to be a modern dung beetle genus that diversified in the Cenozoic, most likely in the Oligocene (approximately 23–33 million years ago) coincident with the expansion of grassland habitats and the radiation of mammals (Darlington 1957; Cambefort 1991b; Davis et al. 2002). This genus of dung beetles is thought to have originated in Africa (Matthews 1972; Hanski and Cambefort 1991; Davis et al. 2002), but today inhabits all continents except Antarctica. Extant species live in a breadth of habitats ranging from desert to tropical wet forest, and they feed on almost every type of dung imaginable.

Onthophagine beetle species are similar enough morphologically that attempts to split the genus have failed (Matthews 1972). Yet, they differ dramatically in the weapons of sexual selection, the horns. Most species of this genus bear enlarged horns, yet these horns exhibit a tremendous diversity of shapes and sizes, and they extend from multiple physical locations on the beetle (Fig. 1). Depending on the species, horns may extend from the base, center, or front of the head and from several locations on the thorax, and although typically expressed only in males, there are a surprising number of *Onthophagus* species with female horns. *Onthophagus*, therefore, provides an ideal system in which to investigate the evolutionary diversification of enlarged weapons of sexual selection. This study focuses primarily on evolutionary changes in the physical location of the horns and secondarily on changes in horn shape.

We carried out a series of phylogenetic analyses for a worldwide sample of *Onthophagus* species using partial sequences from four nuclear and three mitochondrial genes. We use this hypothesis of historical relationships to analyze the evolutionary radiation of horn morphologies. Specifically, we test whether each of the various head and thoracic horns arose only once or multiple times. We also examine the rare instances where females produce enlarged horns, and ask whether these reflect a single or multiple evolutionary event(s), and whether female horns arose in concert with or in isolation from corresponding gains of horns in males. For the most common horn type (horns at the base of the head of males), we examine patterns of transformation of horn shape. Finally, we explore whether transformations in horn morphology are associated with major changes in population density, ecology, or habitat. Combined, our results provide an explicit and comprehensive characterization of the evolutionary divergence of beetle horns. Our phylogeny reveals prolific evolutionary radiation of these exaggerated sexually selected structures. We discuss these results in the context of the natural history of these beetles and suggest ways that sexual selection and ecology may have interacted to generate this extraordinary divergence in animal form.

MATERIALS AND METHODS

DNA Extraction, Amplification, and Sequencing

We sampled DNA from 48 *Onthophagus* species and three outgroups. Onthophagine species are listed in Appendix 1. Outgroups included *Ateuchus* sp. (Coleoptera: Coprinae: Dichotomiini), *Canthidium aurifex* (Coleoptera: Coprinae: Dichotomiini), and *Phanaeus* sp. (Coleoptera: Coprinae: Phanaeini). Aside from deliberate attempts to include taxa from all inhabited continents, taxa were sampled opportunistically (i.e., we included all that we could get DNA from), and randomly with respect to horn morphology. Beetles were placed into either 70% ethanol or, more commonly, into vials two-thirds filled with indicating Drierite silica crystals (Drierite Co., Xenia, OH). Both of these methods preserve DNA strands intact and require no refrigeration during transport to the laboratory. DNA was extracted from whole beetles or, in some large specimens, from only the abdomen. DNA was extracted using the DNeasy Tissue Kit (Qiagen, Valencia, CA).

Using the polymerase chain reaction (PCR) we amplified 219 bases of the nuclear large ribosomal subunit 28S, 441 bases of the nuclear gene 3059 (HMT1 hnRNP methyltransferase-like 4 [*S. cerevisiae*]), 358 bases of the nuclear gene 3089 (ARD1 homolog, N-acetyltransferase [*S. cerevisiae*]), 672 bases of the nuclear gene 8029 (neurofibromin 1), 612 bases of the mitochondrial cytochrome oxidase subunit I (COI), 545 bases of mitochondrial cytochrome oxidase II (COII), and approximately 468 bases of the mitochondrial large ribosomal subunit 16S. Primers (5′-3′) used were: 28SO1 (AGC GGA GGA AAA GAA ACT AAC; designed by D. Bridge, GenBank M11167), 28SD1B (TAG CTT TAG AAG GAG TTT ACC; designed by R. DeSalle, GenBank M11167); 3059fin1F (GGN ATH CAY GAR GAR ATG); 3059fin2F (GAR TGG ATG GGN TAY TG); 3059fin3R (TAR AAN ACN GTY TGY TTC CAR TG); 3089fin1F (GAR AAY TAY CAR ATG AAR TAY TA); 3089fin2F (CAR ATG AAR TAY TAY TTY TAY CA); 3089fin3R (GCR TAR TAY TTN GGY TC); 8029fin4F (CAR GCN AAR ATH TGG GG); 8029fin7R (CAT RCA NGC YTC CAT DAT YTC); LCO 1490 (GGT CAA CAA ATC ATA AAG ATA TTG G); HCO 2198 (TAA ACT TCA GGG TGA CCA AAA AAT CA; Folmer et al. 1994); mtD-18 (CCA CAA ATT TCT GAA CAT TGA CCA; Simon et al. 1994), MTd-
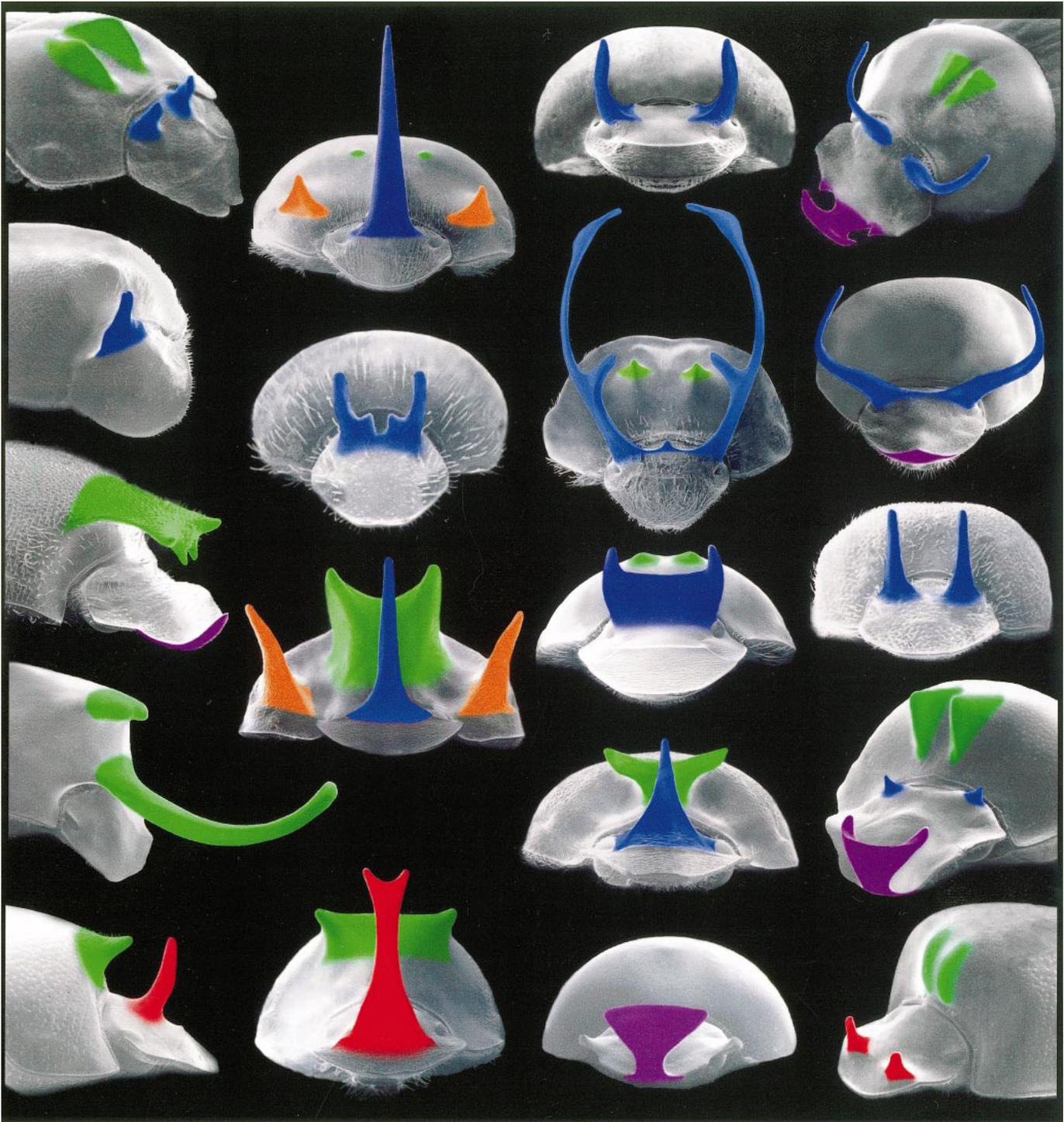
FIG. 1.   Weapon diversity within the beetle genus *Onthophagus*. Head and pronotum shown for 17 species illustrating variation in the shape, size, and physical location of horns. For character mapping, horns were classified as arising from one of five developmental locations: the back of the head (H1, blue), the middle of the head (H2, red), the front of the head (H3, purple), the center of the pronotum (H4, green), or the sides of the pronotum (H5, orange; see text for justification). All individuals are male unless otherwise indicated. Top row: *O. xanthomerus* (female), *O. tersidorsis*, *O. gazella*, *O. xanthomerus*. Second row: *O. nuchicornis*, *O. sugillatus*, *O. rangifer*, *O. taurus*. Third row: *O. hecate*, *O. pentacanthus*, *O. capella*, *O. asperulus*. Fourth row: *O. nigriventris*, *O. ferox*, *O. praecellens*. Bottom row: *O. sagittarius* (female), *O. haagi*, *O. sharpi*, *O. sagittarius*. Horns of *O. tersidorsis* and *O. rangifer* curve back over the body and are shown in an unnaturally erect position to illustrate their form. Photos are scanning election micrographs with artificial color added to illustrate horn locations.

3138 (AGA GCT TCA CCC TTA ATA GAG CAA; modified from C2-N-3661 in Simon et al. 1994); and mtD-34(Sar) (CGC CTG TTT AAC AAA AAC AT; Simon et al. 1994), mtD-32(sbr) (CCG GTC TGA ACT CAG ATC ACG T; Simon et al. 1994). If 16S failed to amplify with these last two primers, mtD-32 sbr was replaced with mtD-29 (GGT CCC TTA CGA ATT TGA ATA TAT CCT; from NADH dehydrogenase subunit 1 in Simon et al. 1994), resulting in a 813-base product.

Products from 28S were purified with QIAquick PCR Purification Kit (Qiagen) and sent to the Murdock Molecular Biology Facility at the University of Montana or the University of Chicago Cancer Research Center to be sequenced. Where possible, COI, COII, and 16S PCR products were purified and sequenced as above. When sequence reactions failed, samples were first cloned using the Original TA Cloning Kit (Invitrogen, Carlsbad, CA), and then sequenced as above. Cloned plasmids were isolated (see Sambrook et al. 1989) and screened for positives using the restriction enzyme *Eco*RI (New England BioLabs, Beverly, MA) following manufacturer's instructions. Genes 3059, 3089, and 8029 were purified using Perfectprep PCR Cleanup 96 kits (Eppendorf, Westbury, CT) and sequenced at Duke University (Cunningham Lab).

Sequences were manually edited using Sequencher 4.0 (Genecodes Corp., Ann Arbor, MI). Ambiguous, or uneditable sequences were amplified and sequenced again, or, where necessary, cloned (see above). Edited sequences were aligned using default settings in ClustalW (Thompson et al. 1994).

Phylogenetic Analyses

We performed maximum likelihood (ML) and six-parameter maximum parsimony (6P) analyses using PAUP 4.0b10 (Swofford 2002), and Bayesian analyses using MrBayes 3.0 (Huelsenbeck and Ronquist 2001).

The best-fit ML model identified by Modeltest (Posada and Crandall 1998) was the general time reversible (GTR; Tavaré 1986) model with substitution frequencies for each of the six nucleotide substitution classes (A ⇒ C, A ⇒ G, A ⇒ T, C ⇒ G, C ⇒ T, G ⇒ T), base frequencies, the percentage of invariant sites, and the shape of the gamma distribution all estimated using ML (Gu et al. 1995; Waddell and Penny 1996). Parameter values were first estimated from the data (using ML to describe the most parsimonious tree; see 6P analyses below), and then fixed for all heuristic and bootstrap ML analyses (Swofford et al. 1996). ML trees were inferred from 10 random addition replicates using TBR branch swapping.

6P analyses also give a different weight to each of the six nucleotide substitution classes based on estimates of their substitution rates (Williams and Fitch 1990; see also Cunningham 1997; Stanger-Hall and Cunningham 1998). Methods for obtaining 6P step-matrices of substitution rates are described in Stanger-Hall and Cunningham (1998). Briefly, for each data partition (see below), we first found the most parsimonious tree (using equally weighted, unordered parsimony) and then used this tree to estimate base frequencies, among-site rate variation, and a matrix of substitution frequencies for all of the substitution classes (R matrix). Substitution frequencies were converted to substitution rates by taking the six values from the R matrix, converting them to proportions, and taking their natural log (for justification, see Cunningham 1997; Stanger-Hall and Cunningham 1998). We used a separate matrix of substitution rates for each of the 12 data partitions (3059 first and second positions; 3059 third positions; 3089 first and second positions; 3089 third positions; 8029 first and second positions; 8029 third positions; COI first and second positions; COI third positions; COII first and second positions; COII third positions; 16S; 28S), and down-weighted five of the partitions (third positions of 3059, 3089, 8029, COI, and COII) to 0.1. For all heuristic and bootstrap 6P analyses, weights were applied using a posteriori stepmatrices under a generalized parsimony framework (Williams and Fitch 1990; Swofford et al. 1996).

We used bootstrapping to assess tree support. 6P bootstrap analyses used TBR branch swapping and 10,000 replicates; ML bootstrapping used TBR branch swapping with a maximum number of 8000 rearrangements and 100 replicates. In pilot runs, we found that 8000 rearrangements were a reasonable compromise between precision and computation time (still more than two weeks on a UNIX system).

We also used the Bayesian method of phylogenetic inference (Rannala and Yang 1996; Mau et al. 1999) as implemented in MrBayes 3.0 (Huelsenbeck and Ronquist 2000). For the Bayesian analyses, we assumed a GTR model with estimated parameter values for base frequencies, proportion of invariant sites, and for the shape of the gamma distribution with six categories. We used the unlink option to estimate parameter values separately for each data partition. We ran 2,000,000 generations of four simultaneous Markov chain Monte Carlo chains and sampled trees every 500 generations. We used four heated chains with a temperature of 0.2 and a swap frequency of 0.1. For burn-in, we discarded trees from the first 250,000 generations (likelihood values stabilized after the first 50,000 generations). For the remaining trees, we computed a majority-rule consensus with the percent of times a clade occurred among the sampled trees as the posterior probability of that clade existing (Huelsenbeck 2000).

Character Mapping

Evolution of horn location.—We grouped horns into five general classes based on their developmental location. Horns at the back of the head extend from the vertex (H1, blue in Fig. 1). These horns result from enlargement of a ridge located on the vertex that runs across the posterior margin of the head, between the eyes. Horns in the middle of the head occur as outgrowths of the frons (H2, red). Horns at the front of the head arise as outgrowths of the clypeus (H3, purple). Clypeal horns range from an upturned elongated lip to broad curved spatulas that extend from the anterior margin of the faceplate. Thoracic horns extend forward from the pronotum, arising either from the center (H4, green) or from the sides (H5, orange) of the pronotal shield. We used both direct observation of specimens and descriptions of horn location from taxonomic keys (listed in Appendix 1) to classify species for horn location. In some taxa horns are visible as rudimentary bumps that are never enlarged into an exaggerated

weapon (these are shown in parentheses in Appendix 1). Because these never develop into full horns, they are not likely to function as enlarged weapons, and we do not include them in our reconstruction of horn evolution here.

Each horn type was mapped on the phylogeny as a two-state character using parsimony and MacClade 4.0 (Maddison and Maddison 1999). Because the typical situation was for males to have horns and females to be hornless, we mapped horns in females as separate characters from horns in males. Mapping female horns separately permitted us to explore whether gains of horns in females occurred independently from gains of horns in males. Thus, the five horn locations were mapped separately for males and females, for a total of 10 horn types.

The robustness of hypotheses of multiple horn origins was tested by comparing the ML tree with trees constrained to alternative hypotheses. Constraint trees were constructed to reflect possible evolutionary histories with only a single gain or loss of each horn type. Constraint trees were fit to the molecular data, and the likelihood scores of these trees were compared with the score of the most likely (i.e., unconstrained) tree using Shimodaira-Hasegawa tests in PAUP 4.0b10 (Shimodaira and Hasegawa 1999; Goldman et al. 2000; Swofford 2002). Significance tests reflect the extent to which the molecular characters support a hypothesis of multiple evolutionary origins of horns.

As a second test for robustness of hypotheses of multiple horn transformations, we averaged the number of estimated gains and losses of horns across all of the 3500 trees encountered by the Bayesian analysis. Because the MrBayes MCMC approach encounters trees in proportion to their posterior probabilities, averaging across all 3500 MCMC trees (after 1000 trees have been discarded for burn-in) provides a conservative estimate of character evolution.

Evolution of horn shape.—Onthophagine horns have changed in shape just as dramatically as they have changed in physical location. Changes in horn shape are most obvious for the most common horn type, male horns at the base of the head (H1, blue in Fig. 1). Thirty species included in this study have male horns at the base of the head (Appendix 1), and these horns exist in 10 distinct shapes. To trace evolutionary changes in the shape of this horn, we excluded all taxa not bearing the horn, scored each species for head horn shape, and mapped this on to the phylogeny as a single character with 10 states. The average number of transformations among horn shapes was calculated using MacClade 4.0 (Chart Changes and Stasis; Maddison and Maddison 1999), and these values were used to reconstruct likely pathways of evolution of horn form. Horns at the other physical locations also appeared to change in shape, but these horn types were not represented in a sufficient number of sampled taxa to merit analyses of shape-evolution.

Evolution of habitat and/or ecology.—To explore possible relationships between the evolution of horn morphology and changes in beetle habitat or ecology, we mapped six additional characters on the phylogeny. These were continent of endemism, two estimates of natural population density (high population density and low population density), and three estimates of habitat use/behavior (nocturnal flight, forest habitat preference, and extreme diet specialization).

Continent of endemism was mapped on the phylogeny as a four-state character (Africa, Eurasia, Australia, America). High population density (not high density, high density) referred to species with extraordinarily large populations. High density taxa are generally the numerical dominants within their native communities, are present at all or most available dung pads in numbers ranging from hundreds to thousands of individuals per pad, and are typically represented by hundreds of individuals in museum collections; many of these species have been highly successful invaders of nonnative habitats as well. Low population density was mapped as a two-state character (not rare, rare) and designated species with characteristically low population densities. These taxa are notoriously difficult to collect in number, are often present at only a small fraction of available dung pads, and are typically represented by only a few individuals in museum collections.

Nocturnal flight behavior (does not fly at night, flies at night) included a number of completely nocturnal taxa, as well as several species (e.g., *O. binodis*, *O. coscineus*, *O. praecellens*, *O. sagittarius*) that fly both by day and at night, and distinguished these taxa from those that fly only during daylight hours. Forest habitat preference (not forest inhabitant, forest inhabitant) distinguished species living inside dense (e.g., lowland tropical) forests from those that inhabit open grassland/pasture habitats.

Finally, most of the taxa included in this phylogeny are diet generalists: they readily come to a variety of dung types, including dung of domesticated animals like horse and cow. However, a few of the included taxa are extreme diet specialists, feeding primarily on carrion (*O. haagi*), rotting fruit (*O. sharpi*), or on dung balls stolen from another species (kleptoparasites; *O. alcyonides*). A few species even cling to the fur of marsupials (e.g., *O. muticus*; Matthews 1972). Diet specialization was mapped as a two-state character (not specialist, specialist).

Species scorings for ecological characters are listed in Appendix 2. For all of these characters, species were scored based on observations of the authors, published taxonomic keys, and correspondence with relevant entomologists.

Concentrated changes tests.—To test for correlated evolutionary changes among mapped discrete characters, we used the concentrated changes test (Maddison 1990) as implemented in MacClade 4 (Maddison and Maddison 1999). Two types of correlated evolution were examined. First, we tested for correlated evolution among the different horn types. Second, we explored whether changes in horn morphology were associated with changes in beetle population density, habitat, or behavior.

Independent contrast analyses.—To analyze the evolution of continuous characters, we employed regression analyses on independent contrasts (CAIC, Purvis and Rambaut 1995). Continuous characters mapped included the total number of horns and the relative length of horns, and each of these was regressed on to our estimate of population density. For these regression analyses, population density was scored as a single, three-state character (-1, 0, 1) reflecting low, intermediate, and high densities (as scored above; Appendix 2). For the horn number versus population density analysis, horn number was the dependent variable and population density

the independent variable. Relative horn length was measured using multiple regression with log total horn length (the $\log_{10}$ of the sum of the lengths of all horns, calculated from species mean values) as the dependent variable, and both log body size (the $\log_{10}$ of the species mean prothorax width) and population density as independent variables. This tested for an effect of population density in explaining the evolution of total horn length, after removing any correlated effects of body size. All regressions of independent contrast values were constrained to have a zero-intercept (i.e., they were forced through the origin; Harvey and Pagel 1991; Purvis and Rambaut 1995).

RESULTS

Phylogenetic Analyses

The aligned 28S data matrix consisted of 219 nucleotide sites, of which 21 were variable and 14 (67%) were parsimony informative. For 3059, the aligned matrix consisted of 441 nucleotide sites, with 139 variable and 101 (73%) parsimony informative; the 3089 matrix consisted of 358 nucleotide sites, with 97 variable and 65 (67%) parsimony informative; the 8029 matrix consisted of 672 nucleotide sites, with 188 variable and 125 (67%) parsimony informative; the COI matrix consisted of 612 nucleotide sites, with 266 variable and 215 (81%) parsimony informative; the COII matrix consisted of 545 sites, with 254 variable and 191 (75%) parsimony informative; and the 16S data matrix consisted of 468 sites, with 158 variable and 115 (73%) parsimony informative. The combined dataset consisted of 837 parsimony-informative characters.

We were unable to obtain sequence data for all seven genes in every species, and for several taxa (e.g., *O. fuliginosus*, *O. lanista*, *O. muticus*) we were only able to sequence a few of the genes (see Appendix 2). To account for missing data, we performed a hierarchical series of phylogenetic analyses, first including taxa with at least five of the seven genes sequenced (20 *Onthophagus* species and one outgroup), then including taxa with at least four of the seven genes sequenced (36 *Onthophagus* species and three outgroups), and finally including all taxa with at least one of the genes sequenced (48 *Onthophagus* species and three outgroups).

ML analysis using the best fit model, TBR branch swapping, and 10 random additions for the taxon subset with the most complete information (at least five of the seven genes sequenced) resulted in a single most likely tree with a score of −ln 17478.47. The ML bootstrap tree (100 pseudoreplications) for this dataset was identical to the consensus tree from the Bayesian analysis and is shown with ML and 6P bootstrap values and Bayesian posterior probability values in Figure 2.

ML analysis for the second subset of taxa (at least four of the seven genes sequenced) resulted in a single most likely tree with a score of −ln 23816.27. The ML bootstrap tree (100 pseudoreplications) for this dataset also was identical to the consensus tree from the Bayesian analysis and is shown with ML and 6P bootstrap values and Bayesian posterior probability values in Figure 3.

Inclusion of all of the taxa (one to seven genes sequenced) resulted in a single most likely tree with a score of −ln
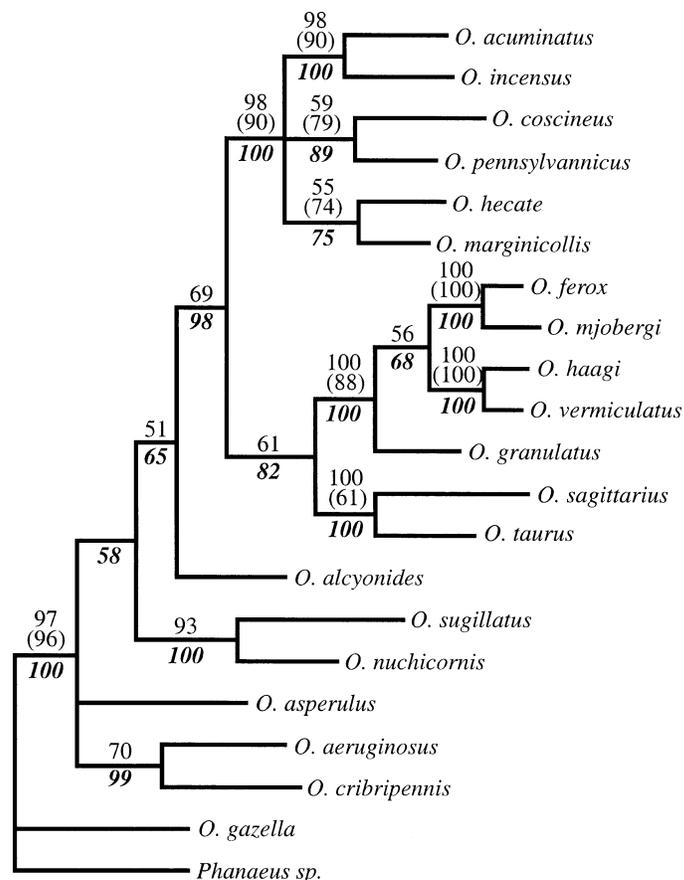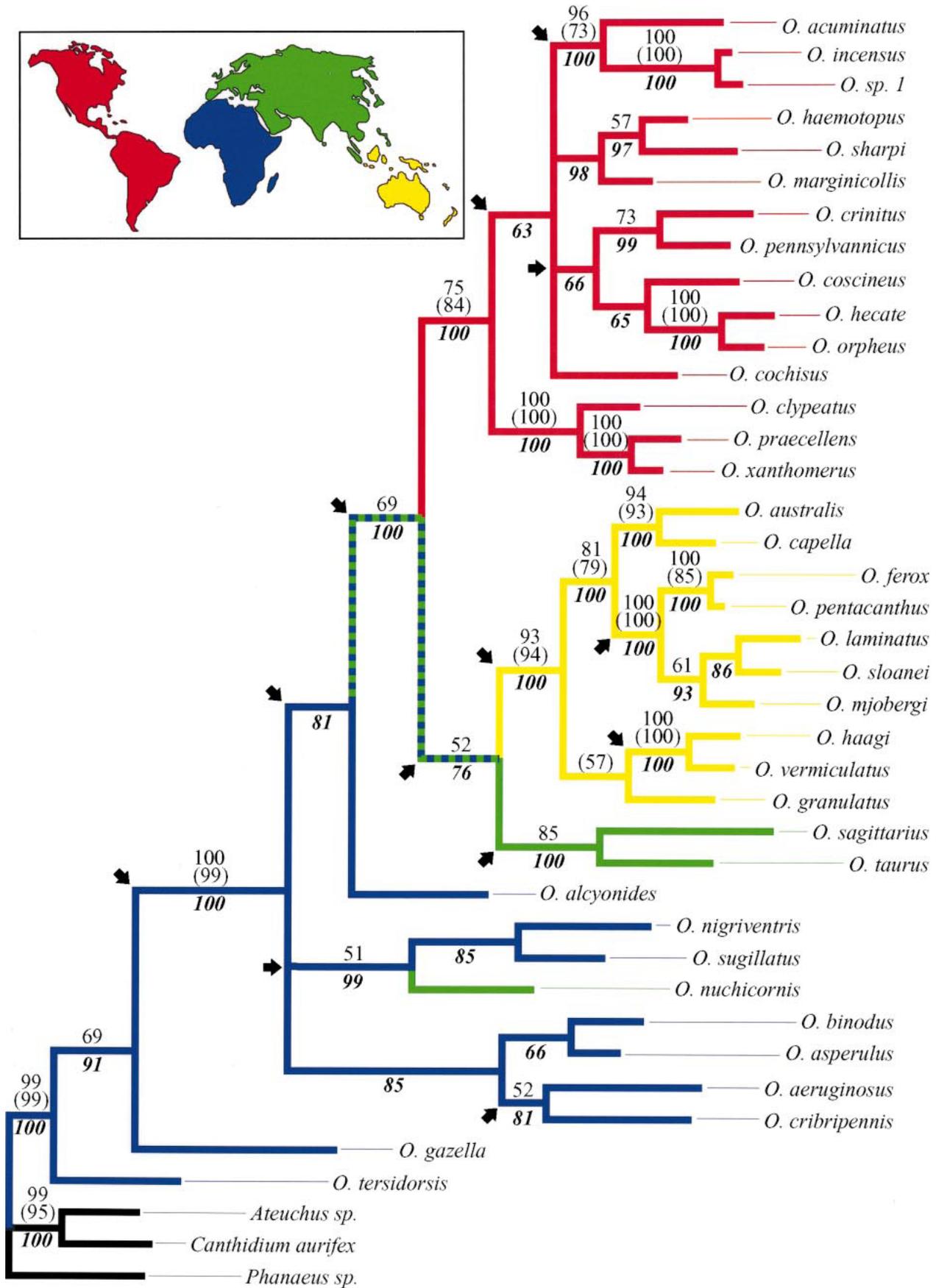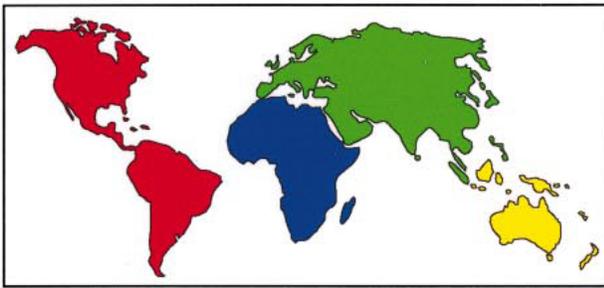

FIG. 2. Maximum likelihood tree for the 20 *Onthophagus* species and single outgroup species with sequence data for at least five of the seven genes (Appendix 2). Maximum likelihood analysis used the best-fit model (GTR + G + I), TBR branch swapping, and 10 random additions (score of best tree = −ln 17478.47). Tree support was estimated in three ways: bootstrap support for 100 pseudoreplicates of the best-fit maximum likelihood model (plain text), bootstrap support for 10,000 pseudoreplicates of six-parameter step matrix maximum parsimony (parentheses), and posterior probabilities of clade occurrence from Bayesian analyses (bold text, italics). See text for justification.

25561.3. This phylogeny is shown fully resolved with 6P bootstrap values and Bayesian posterior probabilities in Figure 4 (ML bootstrap analysis was not feasible due to the large dataset size and high proportion of missing values). Combined, this nested series of phylogenetic analyses provides a robust snapshot of the history of this genus; all three phylogenies agreed well with each other, with the biogeographical distribution of the genus (Fig. 3), and with species groupings of taxa suggested by systematic treatments based on morphological characters (e.g., Boucomont 1932; Matthews 1972).

Evolution of Horn Location: Males

Our data suggest that a horn extending from the base of the head (H1, blue in Fig. 1) of males was ancestral to this genus, and it is possible that horns at this location resulted from only a single initial evolutionary gain (preceding the

O. acuminatus
O. incensus
O. sp. 1
O. haemotopus
O. sharpi
O. marginicollis
O. crinitus
O. pennsylvannicus
O. coscineus
O. hecate
O. orpheus
O. cochisus
O. clypeatus
O. praecellens
O. xanthomerus
O. australis
O. capella
O. ferox
O. pentacanthus
O. laminatus
O. sloanei
O. mjobergi
O. haagi
O. vermiculatus
O. granulatus
O. sagittarius
O. taurus
O. alcyonides
O. nigriventris
O. sugillatus
O. nuchicornis
O. binodus
O. asperulus
O. aeruginosus
O. cribripennis
O. gazella
O. tersidorsis
Ateuchus sp.
Canthidium aurifex
Phanaeus sp.

period included in our phylogeny). Today, head horns are characteristic of males of the majority of *Onthophagus* species. However, our sampling of extant taxa revealed multiple losses of these horns and several possible regains (Fig. 5a). The most parsimonious reconstruction of male head horn evolution required 10 events, with nine losses and one regain (for discussion of the difficulties distinguishing multiple losses from losses and regains, see Cunningham 1999). When an alternative phylogenetic hypothesis involving only a single evolutionary loss of horns was fit to the molecular data, the most likely tree had a −ln likelihood (L) score significantly larger than the unconstrained tree with multiple horn losses (difference in −ln L scores: 666.4, $P < 0.000$; Table 1), indicating strong molecular support for multiple horn losses.

At least two of the inferred evolutionary losses of head horns are also supported by observations of beetle morphology. In both *O. praecellens* and *O. orpheus* (each associated with a proposed loss of head horns in Fig. 5a), males still produce rudimentary bumps at the base of the head (D. J. Emlen, J. Marangelo, and A. Hurt, pers. obs.). The presence of rudimentary horns suggests that full head horns may have been present in ancestral populations, but that expression of this horn type has been largely (though apparently not entirely) turned off in present-day populations.

The most parsimonious reconstruction of evolution of male horns at the center of the head (H2, red in Fig. 1) revealed two gains; Fig. 6a). An alternative hypothesis tree constrained to have only a single gain of horns had a significantly larger −ln-L score than the unconstrained tree with three gains (difference in −ln L scores = 118.8, $P < 0.000$; Table 1), indicating strong molecular support for multiple evolutionary gains of this horn type.

The most parsimonious reconstruction of evolution of horns at the front of the head (H3, purple in Fig. 1) revealed two gains of these horns in males (Fig. 6a). However, a hypothesis tree constrained to have only a single gain was not significantly less likely than the unconstrained tree with two separate gains (difference in −ln L scores = 14.2, $P = 0.217$; Table 1), suggesting that these may reflect a single event.

The most parsimonious reconstruction of male horns on the thorax required nine gains of central thoracic horns (H4, green in Fig. 1), and two gains of lateral (H5, orange) thoracic horns (Fig. 7a). Hypothesis trees constrained to have only a single gain each were significantly less likely than the unconstrained tree with nine and two gains, respectively (central horn: difference in −ln L scores = 466.4, $P < 0.000$; lateral horns: difference in −ln L scores = 160.2, $P < 0.000$; Table 1), indicating strong molecular support for multiple independent gains of these horn types.

Combined, our data suggest multiple losses of the ancestral

horn type (H1), and multiple gains of horns at each of the other four developmental locations (H2–H5), for a total of 25 changes in the physical location of male horns, 15 gains of novel male horn types, and one regain of the ancestral horn type. Collapsing poorly supported branches did not alter the qualitative result of prolific horn diversification: when branches not supported by a ML or 6P bootstrap value of at least 50, or a Bayesian posterior probability of at least 90 were collapsed (using the soft polytomy option in MacClade 4.0), the data still suggested 25 transitions in horn location and at least 11 gains of novel horn types. Averaging across all of the 3500 Markov chain Monte Carlo trees encountered by the Bayesian analysis also did not change the qualitative result, with an average of 25.9 transitions in horn location and 14.8 gains of novel horn types.

Reconstructing male horn evolution revealed a general pattern of weapon escalation (as in Parker 1983; Vermeij 1987). With the exception of three losses of all horns (discussed below), male horns were present throughout the history of this genus. The ancestor of the genus appears to have had a single horn (at the base of the head of males, H1), but horn number increased multiple times, either through a splitting of the original horn into two, or even three (e.g., *O. fuliginosus*) horns at the base of the head (six events), or by the addition of horns at novel locations (15 events), resulting in taxa with up to five different horns. Once additional horn types had been added, it was not uncommon for the ancestral horn type to subsequently be lost (nine events), but these losses of H1 tended to occur only after additional, alternative weapons had already been gained (Concentrated changes test: six of nine losses of H1, $P = 0.009$). Novel horn types (H2, H3, H4, or H5), once gained, were not subsequently lost during the period captured by our phylogeny.

Evolution of Horn Location: Females

The most parsimonious reconstruction of the evolution of horns in females required three gains of horns at the back of the head (H1; Fig. 5b), two gains of horns at the center of the head (H2; Fig. 6b), seven gains of horns at the center of the thorax (H4; Fig. 7b), and one gain of horns at the sides of the thorax (H5; Fig. 7b). Alternative hypothesis trees constrained to have only a single gain of horns at the base of the head (H1), the center of the head (H2), and at the center of the thorax (H4) each were significantly less likely than unconstrained trees with three gains of horns (base of the head: difference in −ln L scores = 196.5, $P < 0.000$; center of the head: difference in −ln L scores = 118.8, $P < 0.000$; center of thorax: difference in −ln L scores = 310.1, $P < 0.000$; Table 1), indicating robust molecular support for multiple gains of these female horn types.

Of the 13 gains of female horns, 10 occurred along the

←

FIG. 3. Maximum likelihood tree for the 36 *Onthophagus* species and three outgroups with sequence data for at least four of the seven genes. Tree construction and node support as in Figure 2. Arrows indicate nodes supported by the previous analysis of 21 taxa (Fig. 2). For this figure only, colors indicate continent of endemism, rather than horn location. Our data corroborate the movement patterns of these beetles proposed by systematists (e.g., Matthews 1972; Hanski and Cambefort 1991; Zunino and Halffter 1997; Davis et al. 2002; see also Villalba et al. 2002), with an African origin of the genus (blue), and subsequent colonizations of Eurasia (green), Australia (yellow), and the Americas (red).
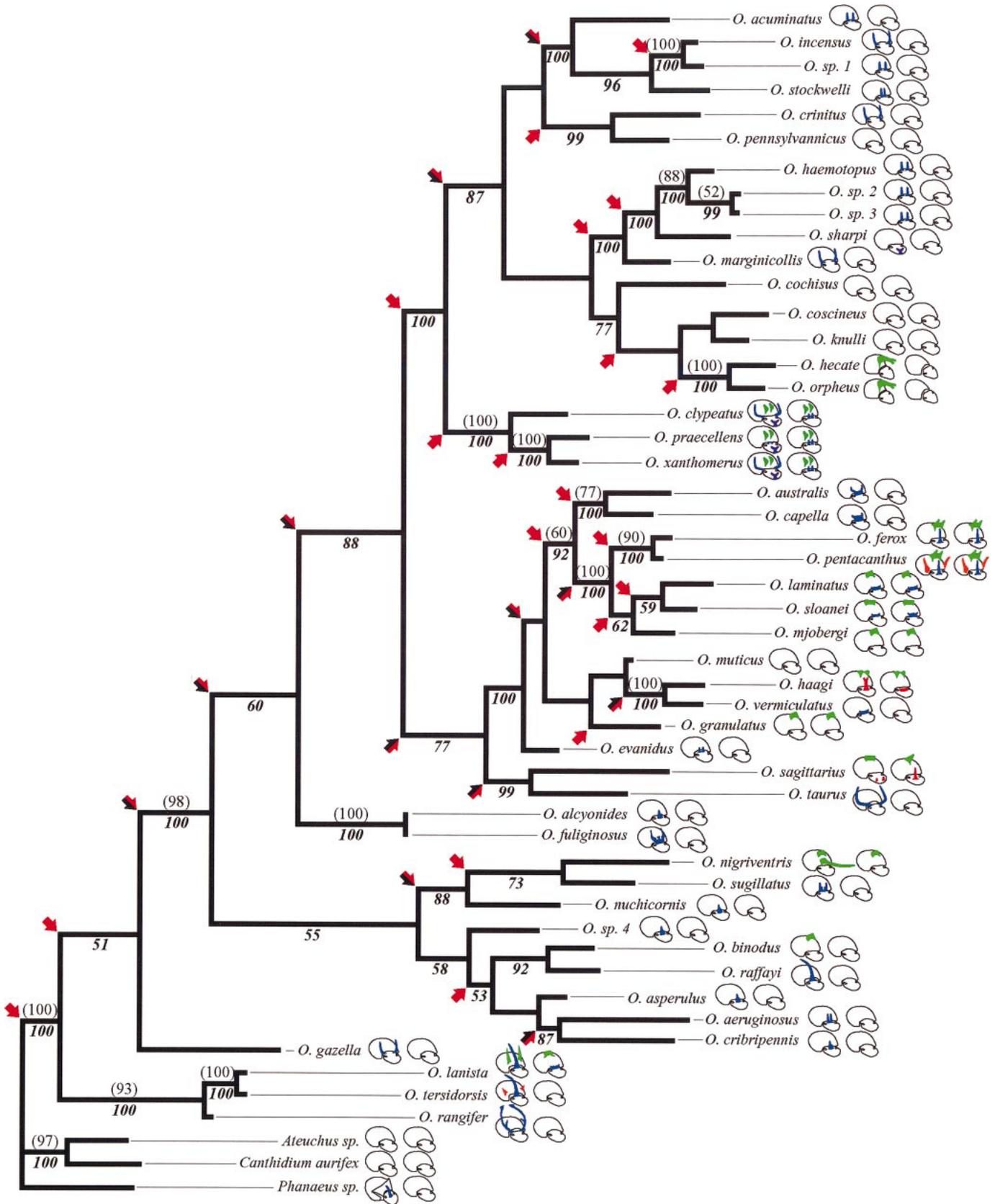
Fig. 4. Fully resolved maximum likelihood tree for all of the 48 *Onthophagus* species included in this study and three outgroups (i.e., with sequence data for at least one of the seven genes; Appendix 2). This single most likely tree resulted from maximum likelihood analysis of 3315 base pairs (837 parsimony informative), a GTR + G + I model, TBR branch swapping, and 10 random additions (score
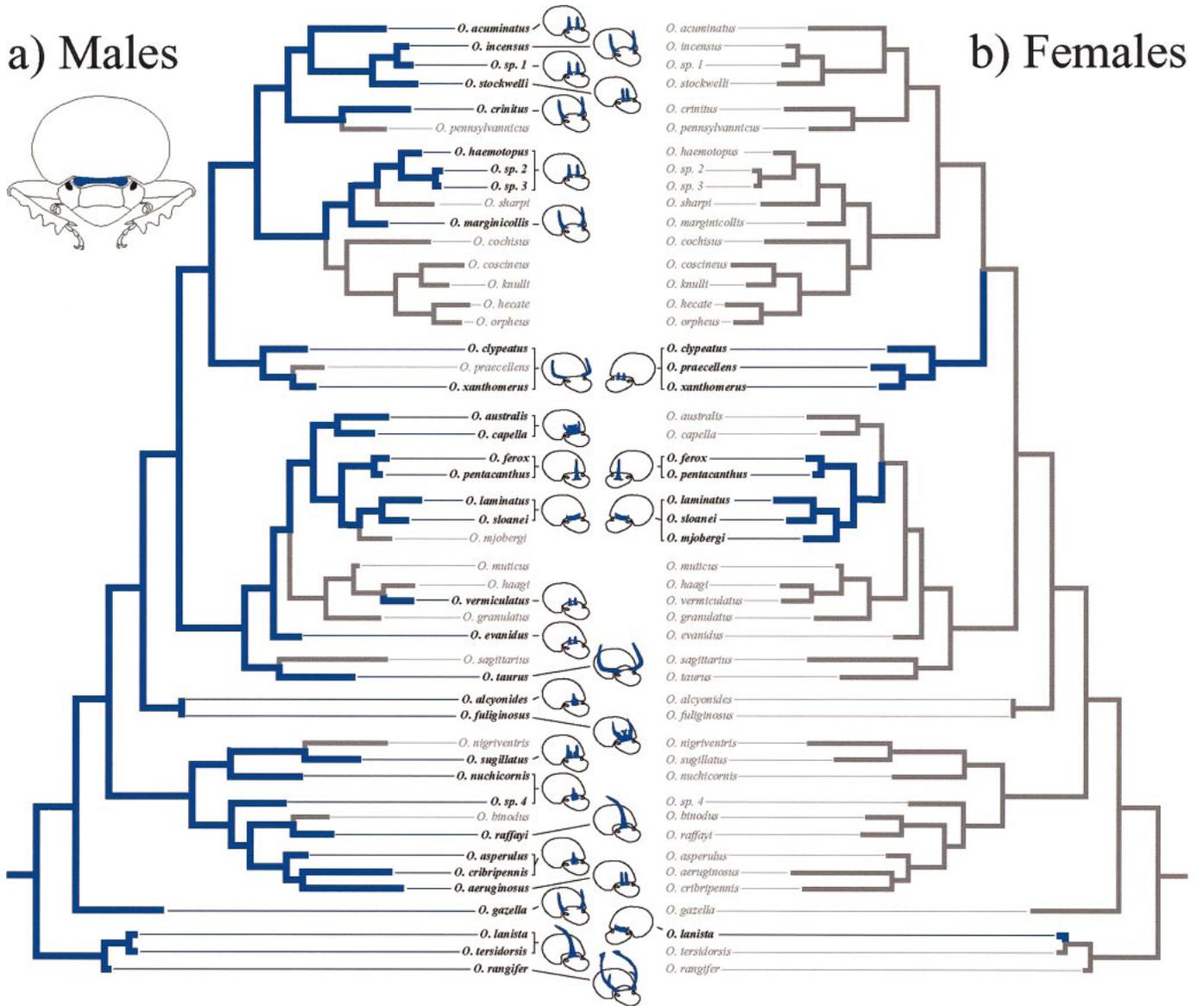
FIG. 5. Evolution of horns at the base of the head (H1) for (a) males; (b) females. The most parsimonious character reconstruction required nine losses and one regain of head horns in males and three gains of head horns in females. Bold blue branches indicate horn presence, and thin gray bars indicate horn absence. Pictures illustrate head horns only (other horn types removed for clarity).

same branches as corresponding gains of the same horn type in males (Concentrated changes test: 10 of 13 gains, $P < 0.000$; Figs. 6, 7). The simultaneous appearance of horns in both males and females suggests that these 10 horn types are expressed independent of sex, that is, the male horn and the female horn represent the same developmental structure. For these cases, we did not consider the origin of female horns to be an additional event from the corresponding gain of horns in males (i.e., we did not include these gains of female horns

in our estimates for the total number of gains and losses of horns).

Of the three female horn types that did not arise simultaneously with the male horn, all involved the appearance of H1 in females of lineages where H1 had long been expressed in males. Only in one lineage did horn types appear in females that were qualitatively different in shape (and relatively larger) than the corresponding horns in males (the H2 and H4 horns of *O. sagittarius*).

←

= −ln 25561.3; see text for methods). Numbers indicate node support from 10,000 parsimony pseudoreplicates (parentheses), and posterior probabilities of clade occurrence from Bayesian analyses (bold italics). Arrows indicate nodes supported by phylogenetic analysis of nested subsets of 39 (red; Fig. 3), or 21 (black; Fig. 2) taxa (see text for justification). Drawings illustrate the horn morphologies of males (left) and females (right) of each included species.

TABLE 1. Tests for multiple transformations of beetle horns. For each horn type, alternative phylogenetic hypotheses were constructed and fit to the molecular data. Likelihood scores were compared between the most likely tree (no constraints enforced), and trees constrained to involve fewer horn transformations, using Shimodaira-Hasegawa tests in PAUP. Significant differences in −ln likelihood scores illustrate robust molecular support for hypotheses of multiple horn evolution.

Horn location	Hypothesis	−ln L	Difference in −ln L	P
Males				
H1 (vertex)	most likely tree (nine losses, one gain)	25561.3		
	alternative hypothesis (one loss, no gains)	26227.7	666.4	<0.001
H2 (frons)	most likely tree (two gains)	25561.3		
	alternative hypothesis (one gain)	25680.2	118.8	<0.001
H3 (clypeus)	most likely tree (two gains)	25561.3		
	alternative hypothesis (one gain)	25575.5	14.2	0.217
H4 (medial pronotum)	most likely tree (nine gains)	25561.3		
	alternative hypothesis (one gain)	26027.8	466.4	<0.001
H5 (lateral pronotum)	most likely tree (two gains)	25561.3		
	alternative hypothesis (one gain)	25721.5	160.2	<0.001
Females				
H1 (vertex)	most likely tree (three gains)	25561.3		
	alternative hypothesis (one gain)	25757.9	196.5	<0.001
H2 (frons)	most likely tree (two gains)	25561.3		
	alternative hypothesis (one gain)	25680.2	118.8	<0.001
H3 (clypeus)	most likely tree (no gains)	25561.3		
H4 (medial pronotum)	most likely tree (seven gains)	25561.3		
	alternative hypothesis (one gain)	25871.5	310.1	<0.001
H5 (lateral pronotum)	most likely tree (one gain)	25561.3		

Evolution of Horn Shape

Onthophagine horns varied in shape as well as physical location. Of the 10 recognized horn types, only male horns at the base of the head (H1) were present in enough of the taxa to permit explicit analyses of horn-shape evolution. Head horns were present in males of 30 of the included *Onthophagus* species (Appendix 1), and this horn type was manifest in 10 distinct shapes (Fig. 8). Mapping horn shape onto the phylogeny and charting the average frequencies of horn-shape transformation suggested that the ancestral horn shape (a single triangular horn arising from the center of the vertex) radiated into at least seven variant forms and that several of these then subsequently radiated into additional forms (Fig. 8). Five of these transformations involved a splitting of the original horn into a pair of horns, and in one case the ancestral horn split into a row of three horns.

Horn Evolution and Habitat/Ecology

Gains of horns.—We predicted that population densities would affect the number of encounters between rival males, and hence, the intensity of sexual selection. Specifically, we predicted that high population densities would be associated with stronger sexual selection and increased investment into weaponry. Independent contrast analyses of continuous horn traits supported this prediction: both total horn number and total horn length were significantly correlated with evolutionary increases in population density (horn number vs. population density: $r = 0.448$, $F = 10.545$, $P = 0.002$, $N = 43$ contrasts; horn length vs. population density: $r = 0.362$, $F = 29.518$, $P = 0.000$, $N = 29$ contrasts).

We then treated high and low population densities as separate two-state characters and tested for correlations with gains and losses of specific horn types. Gains of horns were not associated with low population densities (Table 2). However, eight of the 16 gains of male horns occurred in beetle lineages associated with extraordinarily high population densities (Concentrated changes test: $P = 0.017$; n.s. with Bonferroni correction for multiple comparisons; Table 2), and this pattern depended on horn type. Specifically, gains of thoracic horns (H4, H5) were positively correlated with high population densities (seven of 11 gains, $P = 0.003$), whereas gains of head horns (H1, H2, H3) were not (one of five gains, $P = 0.762$; Table 2). A similar but nonsignificant trend was also observed with gains of female horns (Table 2).

Gains of male head horns (H1, H2, H3) appeared to be associated with lineages inhabiting forests, but this trend was not significant (four of five gains, $P = 0.059$; Table 2). There were no additional detectable associations between ecological variables and gains of male or female horns.

Losses of horns.—Only the ancestral horn type (H1) was lost in this sample of the genus, and this horn appears to have been lost nine separate times (Fig. 5a). Losses of head horns (H1) were correlated with one aspect of beetle ecology: evolutionary shifts from diurnal to nocturnal flight behavior (seven of nine losses of head horns, $P = 0.000$; Table 2). All three complete losses of horns (i.e., gains of hornlessness) also occurred in lineages with nocturnal flight behavior, but this pattern was not significant after Bonferroni correction for multiple comparisons (Table 2).

We detected two additional patterns. First, there was an association between open/pasture habitats and high population densities. Most of the sampled New World taxa (red in Fig. 3) inhabit forests. However, there were five transitions within this clade from forests to open pastures, and four of these coincided with the evolution of extremely high population densities (four of five losses of forest habitat preference, $P = 0.000$). Second, there was an association between
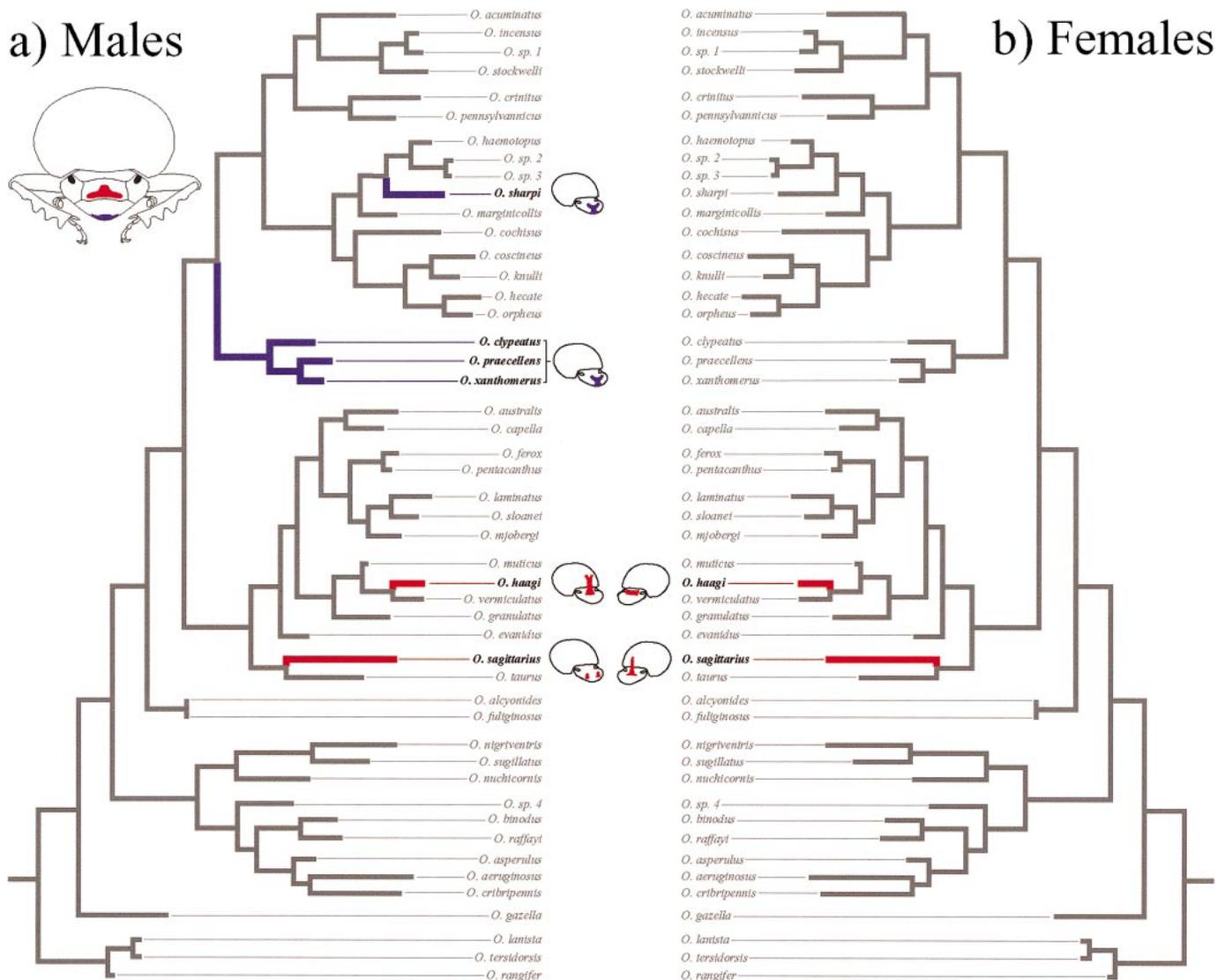
FIG. 6. Evolution of horns at the middle (H2, red) and front (H3, purple) of the head for (a) males; (b) females. For horns at the center of the head (H2), the most parsimonious character reconstructions required two gains in males and two gains in females. For the clypeal horns (H3), the most parsimonious reconstruction required two gains in males and no gains in females. Branch coding as in Figure 5, and colors as in Figures 1 and 4. Pictures illustrate relevant horns only (other horn types removed for clarity).

low population densities and the evolution of extreme diet specialization. Four of the five times diet specialization evolved, it arose in a lineage that also had unusually low population densities (four of five gains of diet specialization, $P = 0.001$). This latter pattern may reveal an artifact of sampling: taxa that specialize on an unusual food resource may only occasionally turn up in dung, and may therefore be underrepresented in museum collections (and erroneously scored as rare). For example, *O. sharpi* only occasionally gets collected at animal dung and is rare in most collections. However, this species occurs under almost all rotting *Gustavia* fruits, and it is not uncommon to find 20 or more individuals under a single fruit (J. Marangelo, pers. obs.).

Summary: Horn Macroevolution in Onthophagus

Combined, our study suggests a multitude of transformations of beetle horns: a sampling of only 48 *Onthophagus*

species yielded at least 25 changes in the physical location and sex-specific expression of horns, including at least 15 independent gains of novel horn types and one regain of an ancestral horn type. These estimates of horn divergence are robust to uncertainty in our tree topology (collapsing unsupported nodes and averaging across the 3500 trees encountered by the Bayesian analysis). Furthermore, our analyses underestimate total weapon divergence, because we discount situations where gains of horns in males and females might reflect the same evolutionary event and because our numbers include only changes in the physical location of horns. Location encompasses just one of the major avenues by which beetle horns evolve. Onthophagine horns have also changed tremendously in both shape (Fig. 8) and overall size, and inclusion of these variations would have increased our numerical estimates of horn divergence substantially. Clearly, the exaggerated weapons of sexual se-
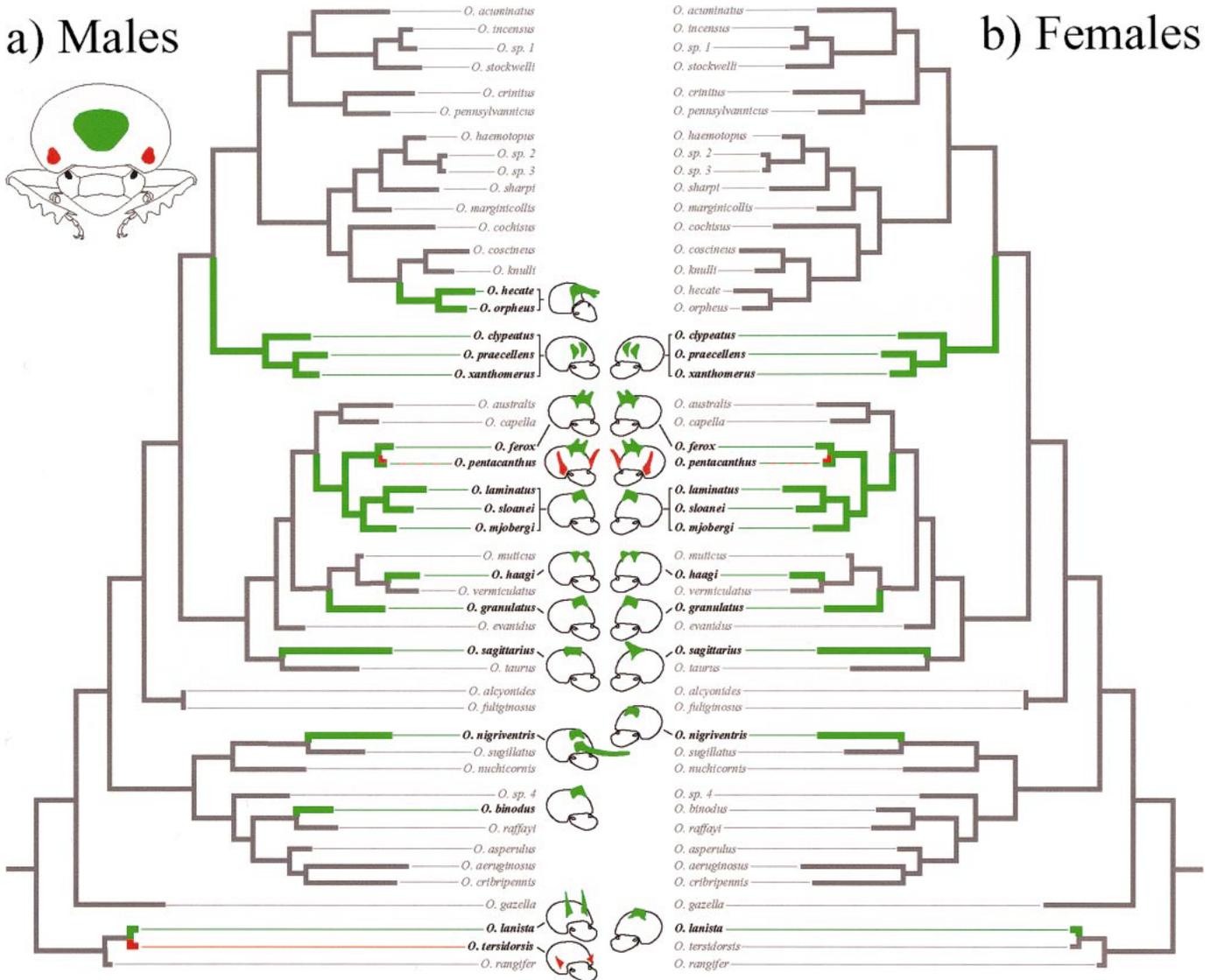
FIG. 7. Evolution of horns at the center (H4, green) and sides (H5, orange) of the thorax for (a) males; (b) females. The most parsimonious character reconstructions required nine gains of horns at the center (H4) and two gains of horns at the sides (H5) of the thorax in males and seven gains of horns at the center and one gain of horns at the sides of the thorax in females. Branch coding as in Figures 5 and 6, and colors as in Figures 1 and 4. Pictures illustrate thoracic horns only (other horn types horns removed for clarity).

DISCUSSION

Sexual selection is credited with the evolution of nature's most fantastic animal morphologies (Darwin 1871; Richards 1927; Thornhill and Alcock 1983; Andersson 1994). Even before Darwin included horned beetles in his treatise on sexual selection (Darwin 1871), the exaggerated shapes of beetle horns had captured the fancy of biologists (Drury 1770; Fischer 1823; Castelnau 1840; Burmeister 1847; Bates 1863; Walsh 1864; Wallace 1869), and this fascination has persisted ever since (e.g., Paulian 1935; Beebe 1944; Arrow 1951; Ratcliffe 1977; Eberhard 1982; Enrodi 1985). Clearly, one focus of this interest has been the extreme sizes of horns in these animals. Yet, it has also been obvious throughout that

beetle horns were impressive not just for their size but also for their variability. Even a superficial glance at beetle horns suggests that they must have evolved independently numerous times and must have undergone extensive macroevolutionary radiations in form (e.g., Arrow 1951; Eberhard 1980; Enrodi 1985). Here, we make this analysis explicit.

We bring a DNA sequence-based phylogenetic hypothesis to bear on the question of beetle horn evolution. We show that even within a single beetle genus, horns display prolific diversification. Sampling of a mere 48 species (~2% of the genus) reveals more than 25 gains and losses of exaggerated horns, and these estimates focus only on evolutionary changes in horn location; inclusion of changes in the size and shape of beetle horns would increase our estimated number of transformations considerably (e.g., Fig. 8). In the following sections, we provide a context for considering the evolutionary
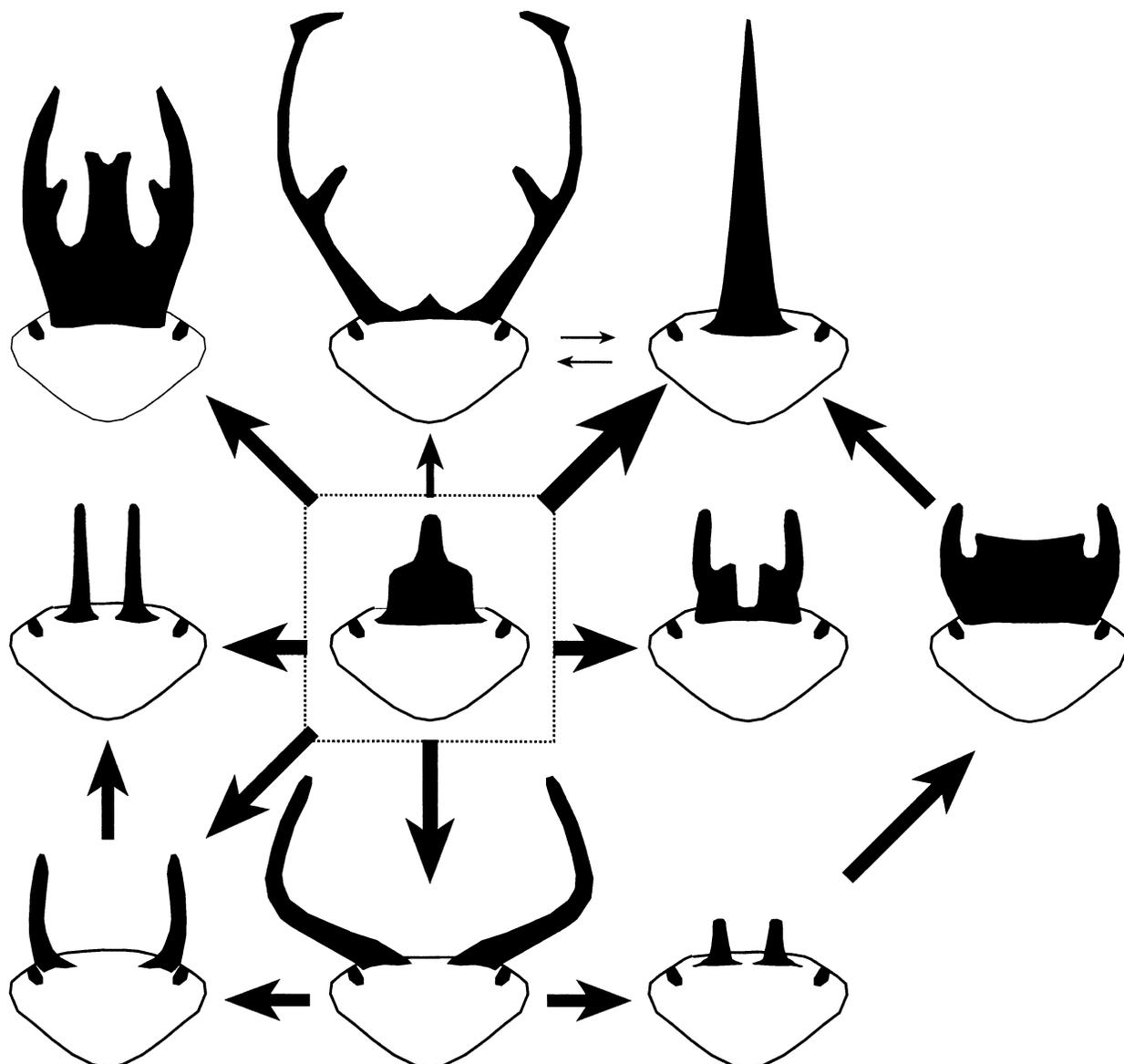
FIG. 8. Evolutionary changes in the shape of male head horns (H1). Arrows indicate pathways of horn evolution, and arrow thickness reflects average frequencies of change along each path (estimated by mapping horn shapes onto the phylogeny using MacClade 4.0). Frequencies were 0.333 (thinnest arrow), 0.667, 1.000, and 1.667 (thickest arrow).

diversification of beetle horns based on the natural history and biology of dung beetles, and we use our phylogeny to provide preliminary tests of one of the proposed mechanisms of weapon divergence.

Natural History of Onthophagus beetles

A variety of historical events contributed to the overwhelming evolutionary success of the Coleoptera (e.g., Mitter et al. 1991; Farrell 1998). One of these was the colonization of animal dung (Halffter and Matthews 1966; Halffter and Edmonds 1982; Hanski and Cambefort 1991). Dung is a nitrogen-rich larval diet, but it is also patchily distributed and highly ephemeral; historically, all dung beetles faced intense competition for this food resource (Hanski and Cambefort 1991). Today, the most species-rich dung beetle clades em-

ploy one of two behavioral strategies to circumvent this competition. Most dung beetle species either roll balls of dung away from the central source (ball rollers; Canthonini, Eucraniini, Eurysternini, Gymnopleurini, Scarabaeini, and Sisyphini), or they dig tunnels into the soil directly beneath dung and bury dung below ground (tunnelers; Coprini, Dichotomiini, Oniticellini, Onitini, Onthophagini, and Phanaeini). Both of these behaviors physically remove dung from the source, and sequester it away from competition while larvae develop (Halffter and Matthews 1966; Halffter and Edmonds 1982; Cambefort 1991a; Cambefort and Hanski 1991).

Onthophagus is a genus of tunneling dung feeders (Halffter and Edmonds 1982; Cambefort 1991a; Cambefort and Hanski 1991; Villalba et al. 2002). Beetles fly to pieces of dung and dig immediately down into the soil below. Tunnel excavation

TABLE 2. Concentrated changes tests for correlated evolution between horns and five relevant aspects of beetle ecology. Each comparison tests whether changes in horn morphology were significantly concentrated on branches of the phylogeny having a designated ecological state (e.g., nocturnal flight) using MacClade 4.0 (see text for justification and methods). Numbers indicate how many of the observed changes in horn morphology occurred on branches with the designated ecological state; adjacent numbers (in parentheses) indicate probability values given parsimony reconstructions of changes in the ecological character. Bonferroni corrections for P-values (α = 0.05): within-category[a–c], 0.003; overall, 0.001. Asterisk indicates significance.

	High pop. density	Low pop. density	Flies at night	Forest habitat	Diet specialist
(a) Gains of male horns					
Head horns: H1,H2,H3	1/5 (0.762)	1/5 (0.682)	2/5 (0.459)	4/5 (0.059)	2/5 (0.020)
Thorax horns: H4,H5	7/11 (0.003)*	1/11 (0.947)	4/11 (0.428)	3/11 (0.921)	1/11 (0.503)
Any horns: H1,H2,H3,H4,H5	8/16 (0.017)	1/16 (0.993)	6/16 (0.376)	5/16 (0.862)	2/16 (0.325)
(b) Losses of male horns					
Head horns: H1	3/9 (0.134)	1/9 (0.901)	7/9 (0.000)*	3/9 (0.770)	1/9 (0.439)
All horns: (i.e., hornless)	1/3 (0.548)	1/3 (0.461)	3/3 (0.017)	1/3 (0.816)	1/3 (0.139)
(c) Gains of female horns					
Head horns: H1,H2,H3	2/5 (0.338)	1/5 (0.671)	2/5 (0.438)	2/5 (0.664)	1/5 (0.260)
Thorax horns: H4,H5	5/8 (0.056)	1/8 (0.876)	3/8 (0.481)	2/8 (0.896)	1/8 (0.506)
Any horns: H1,H2,H3,H4,H5	7/13 (0.015)	2/13 (0.848)	5/13 (0.330)	4/13 (0.861)	2/13 (0.199)

is generally carried out by females (Fabre 1899; Halffter and Edmonds 1982; Emlen 1997; Hunt and Simmons 1998; Moczek and Emlen 2000; Hunt et al. 2002). Once a tunnel has been formed, females pull fragments of dung down to the ends of the tunnel chambers (sometimes with the assistance of large males; Emlen 1994; Hunt and Simmons 1998; Moczek 1998; Hunt and Simmons 2000; Hunt et al. 2002) and fashion them into dense, oval masses called brood balls (Halffter and Edmonds 1982). A single egg is placed in each of the brood balls, and larvae develop in isolation within these underground food masses (Main 1922; Halffter and Edmonds 1982).

Several aspects of their biology appear to have predisposed these beetles to high rates of speciation. First, a history of intense competition for ephemeral food resources may have favored specializing on novel dung sources (Hanski and Cambefort 1991; Davis and Sutton 1997), potentially leading to habitat isolation. Today, many *Onthophagus* species show strong and specific dung preferences, and extant species feed on a tremendous breadth of dung sources (e.g., deer, antelope, marmot, horse, monkey, tapir, agouti, elephant, packrat, kangaroo, prairie dog, sloth, toad); a few species have even colonized fruit, fungi, and carrion (Howden and Cartwright 1963; Howden and Young 1981; Davis and Sutton 1997).

Second, breeding behavior is confined to the food source: all mating takes place inside tunnels beneath dung. Large, horned males fight to guard entrances of burrows containing females, while small hornless males employ an alternative sneaking tactic (Emlen 1997, 2000; Moczek and Emlen 2000). Both tactics revolve around methods of getting inside tunnels to mate with females as they provision burrows and lay eggs. Because breeding occurs at the food source, beetles colonizing a novel dung type would be more likely to reproduce with other colonists of the novel diet than they would be to breed with members of their source population, a form of food-based assortative mating.

Thus, the capacity to colonize novel dung sources, combined with reproductive behaviors that link breeding with the food source, may have facilitated the evolution of reproductive isolation and species formation in this genus of beetles.

With this as a backdrop, we can begin to examine the evolution of horns.

Why Do So Many Onthophagus Species Have Horns?

Males of a great many insect species battle with rival males over reproductive access to females (Thornhill and Alcock 1983). Yet most of these animals do not bear horns. In beetles, the ecological situation that best separates species with horns from those without horns is the confinement of breeding behavior to a restricted space such as a subterranean burrow. The mechanical advantage of horns appears to arise when males battle over a confined space, and dung beetle burrows meet this criterion (Emlen 2000). The linear, cylindrical nature of the burrows renders the resident females logistically defendable: by blocking tunnel entrances, males can keep rivals at bay and maintain exclusive access to the females inside.

Of the two principle categories of dung beetles, the tunnelers and the ball-rollers, exaggerated male horns are almost entirely confined to the tunnelers. Ball-rolling males frequently fight over access to females, but these fights do not occur inside burrows, and these males rarely, if ever, bear horns. Even most nondung beetles with horns use them in fights over burrows, though the burrows need not be subterranean (e.g., *Podischnus agenor* [Coleoptera: Dynastinae] males fight over tunnels hollowed out of sugar cane stems; Eberhard 1979, 1982). Exceptions to this rule tend to include species that fight over otherwise similarly linear and restricted substrates (e.g., emergent plant shoots or branches, as in *Golofa porteri* [Coleoptera: Dynastinae]; Eberhard 1978). Thus, the confinement of breeding to a localized and economically defendable burrow (or branch) appears to be an evolutionary prerequisite that sets the stage for subsequent elaboration and exaggeration of male weaponry.

In *Onthophagus*, tunneling behavior is ubiquitous. Despite an incredible breadth of dung sources used by species in this genus, and a corresponding breadth of physical habitats occupied (tropical wet forests to desert), all of these beetles do basically the same thing: when they find dung, females dig

burrows into the soil below, and males guard the entrances to these burrows.

Males of *Onthophagus* species use their horns in fights with rival males inside these burrows. Males brace themselves against tunnel walls, and use horns to block, pry, or dislodge intruder males (Emlen 1994, 1997, 2000; Moczek and Emlen 2000; D. J. Emlen and J. Marangelo, pers. obs.). Success in these fights is influenced by the relative length of the horns of contestant males: males with longer horns win (Emlen 1997; Moczek and Emlen 2000; Hunt and Simmons 2001; for similar results from other dung beetle genera see Otronen 1988; Rasmussen 1994). Because success in these encounters depends on horn size relative to the horn sizes of rival males, beetle populations likely experience continuous directional sexual selection for escalated investment in weapons (as described in Parker 1979, 1983; West Eberhard 1983). Results from the present study support this premise: with only three exceptions, horns were present in some form in all lineages and for the entire period covered by this phylogeny, and horn sizes repeatedly reached outrageous proportions (e.g., in three separate lineages [*O. nigriventris*, *O. raffrayi*, and *O. tersidorsis*] horn lengths exceeded the total length of the rest of the body). Males also increased investment in weapons by adding new horns to their arsenal. Horn number increased at least 21 separate times, resulting in species with two, three, or even five different horn types.

Thus, we suggest that tunneling behavior has had at least two profound influences on these dung beetle populations: it may have contributed to speciation (by coupling breeding with the food resource), and it may also have consistently generated intense directional sexual selection for large and numerous weapons. Males in all of these species are likely to benefit from bumps or knobs that provide friction or add leverage in contests or that simply block more of the tunnel corridor. Because longer or larger horns are better than shorter horns for blocking or prying, rapid enlargement of the sizes of these bumps or knobs is likely to ensue. Consequently, the tunneling behavior characteristic to *Onthophagus* may explain why so many of these species bear horns, and also why these horns attain such outrageous proportions. But why are the shapes and physical locations of these horns so diverse?

Why Have Beetle Horns Diverged in Form?

The most difficult question to address in these beetles is why have horns evolved so many different times, and in so many different locations on the animal? Figure 5 suggests that ancestors of this genus possessed a single horn that extended from the base of the head. Yet, it is clear that this head horn has been lost repeatedly (our data suggest nine losses and one regain; Fig. 5a, Table 1), and it has changed considerably in shape (Fig. 8). It is also clear from our phylogeny that numerous additional horn types have arisen (Figs. 6, 7), resulting in a mixture of species with everything from no horns to multiple horns, and all possible combinations in between (Fig. 1). Extant horn types do not represent a small number of conserved, rare events. Instead, our data portray a history fraught with changes in horn morphology, so that today even closely related species differ markedly in the

TABLE 3. Alternative explanations for weapon diversification in beetles. For all of these, it is assumed that directional sexual selection favors rapid enlargement of bumps or ridges into functional horns and that arms races escalate this process (see text for justification). The hypotheses listed below describe mechanisms that could have led to divergence in the form that these weapons took; that is, which regions of cuticle became enlarged into horns and which horns, if any, were lost.

I. Habitat-independent sexual selection
  A. Chance events led to divergence in weapon morphology.
    – Founder events, drift, and population bottlenecks altered the genetic composition of populations, affecting which horn types were present and shifting the trajectory of horn evolution.
  B. Novel horns had a performance advantage.
    – Males with novel horns or novel combinations of horns won contests more frequently than males with traditional horns, generating disruptive selection on horn morphology.
    – Chance events determined which new horn morphologies arose within any population.
II. Habitat-dependent sexual selection
  A. The relative *performance* of horn types differed depending on the habitat/ecology of species.
    – Some horn types functioned better than others within each environment.
    – Colonization of new or different environments, combined with selection for improved fight performance, favored new types or combinations of horns.
  B. The relative *costs* of horn types differed depending on the habitat/ecology of species.
    – Some horn types cost less than others within each environment.
    – Colonization of new or different environments, combined with selection to minimize the costs of horn expression, favored new types or combinations of horns.

shape and form of their weapons. What selective factors may have led to this radiation in weapon morphology?

Hypotheses I and II: habitat-independent sexual selection. One possibility is that horn morphologies are largely neutral with respect to habitat. This could work in two ways. First, ecological factors leading to genetic divergence among populations, coupled with chance events affecting the relative frequencies of horn morphologies, could have generated the diversity in form observed today (Table 3, Ia). We have already mentioned that these beetles may have been especially prone to geographic isolation as colonists capitalized on novel dung sources, and it is possible that founder events associated with these invasions of new habitats resulted in chance alterations to weapon morphology. Thus, intense sexual selection for large protrusions (of any kind), coupled with frequent population bottlenecks and chance events, could have generated interspecific diversity in weapon form (see West Eberhard 1983).

A second manifestation of habitat-independent horn diversification may have occurred if novelty, per se, was favored in male contests (Table 3, Ib; West Eberhard 1983). In this case, chance events generating new horn types could have led to macroevolutionary shifts in horn morphology, as novel horns arose and replaced existing horn forms. In both of these scenarios, evolutionary transitions in horn morphology are predicted to occur independent of habitat or ecol-

ogy—a prediction that appears largely borne out, at least by the ecological variables we have been able to include so far (Table 2).

Hypothesis III: habitat-dependent selection on horn function.—A third possibility is that the different horn types function best in different habitats (Table 3, IIa). Sexual selection for fighting performance (i.e., for the function of the horns) could have favored shifts from one horn type to another if there were differences in the utility of horns across environments, that is, if one horn type functioned better than others. Habitat heterogeneity for which horn performed best could then have led to diversity in horn morphology: as beetles colonized new and different habitats, sexual selection favored new and different weapon forms.

This perspective has been applied to a variety of other animals to explain diversity in secondary sexual structures. Ornaments in male fish, for example, may stand out from backgrounds differentially depending on the characteristics of each specific stream habitat. In this situation, female preferences for conspicuousness may have generated habitat-associated divergence in ornament form (Endler 1980, 1992; Endler and Houde 1995; Boughman 2001). Similarly, for ungulates, Geist (1966, 1978) suggested that changes in both habitat and body size led to shifts in the style of male fighting, and that this favored divergence in antler shape (Kitchener 1985; Lincoln 1994; Lundrigan 1996).

To apply this logic to beetles, we must consider the habitats within which horns function. Specifically, we need to consider why horn types functioning best in one environment might differ from those that function best in other environments.

Onthophagine species certainly inhabit a breadth of environments. As already mentioned, they reside on six continents, feed on a wide array of food sources, and inhabit everything from tropical wet forests to grasslands, woodlands, pastures, and desert. Yet, all of this variation may be irrelevant to the function of beetle horns. Irrespective of continent, climate or habitat, when these beetles find dung they all do the same thing: they dig burrows into the soil below, and fights using horns take place inside these underground burrows. Thus, the immediate ecological context most relevant to horn function (i.e., a cylindrical tunnel in the soil) is practically uniform across species, and is therefore not likely to be a primary factor selecting for divergence in horn morphology. It is not obvious, at present, how habitat-specific selection on the function of horns could favor diversity in horn location, and further studies will be needed to better explore this possibility.

Hypothesis IV: habitat-dependent selection on horn costs.—A fourth possibility focuses on the costs, rather than the function (or benefits), of one horn type over another (Table 3, IIb; Emlen 2000, 2001). Horns may incur costs differentially across habitats, with some horn types being more expensive than others in each ecological situation. In this case, selection to minimize the costs of horn expression could have favored evolutionary shifts in horn location.

Aspects of male guppy color patterns and male cricket songs have been shaped by a selective balance between attracting females and attracting predators or parasitoids (Endler 1980, 1983, 1992; Zuk et al. 1993, 1998; Endler and

Houde 1995; Rotenberry et al. 1996; Zuk and Kolluru 1999). Geographic variation in the risk of predation affects this balance and may have favored very different ornaments or songs in each of the habitats (Endler 1980, 1983; Zuk et al. 1993; Endler and Houde 1995; Rotenberry et al. 1996). Similarly, aerodynamic costs of avian tail ornaments vary with habitat, and this appears to have influenced the directions of male ornament evolution in birds (Balmford et al. 1994; Møller 1995; Johnson 1999; Møller and Hedenström 1999). In all of these situations, costs of the structures, rather than (or in addition to) benefits, drive divergence in morphology.

Beetle horns are very expensive to produce (e.g., Hunt and Simmons 1997; Nijhout and Emlen 1998; Emlen 2000, 2001). One prominent cost of horns appears to result from allocation trade-offs arising during beetle development: animals with enlarged horns also have stunted versions of other structures, including antennae, eyes, and wings (Rensch 1959; Kawano 1995, 1997; Nijhout and Emlen 1998; Emlen 2000, 2001). Remarkably, horns do not stunt all traits equally. Instead, horns primarily impact the growth of physically adjacent or nearby structures. This means that beetle horns can have very different functional costs, depending on their physical location on the animal (Emlen 2000, 2001). Horns developing on the thorax (H4, H5) are likely to reduce the efficiency of flight, since they reduce the relative sizes of wings. Horns developing at the middle or front of the head (H2, H3) are likely to impact the olfactory capabilities of males, as they lead to reductions in the relative sizes of antennae. Horns developing at the back of the head (H1) probably impair vision, because they reduce the relative sizes of eyes. Because the nature of the costs differ from one horn type to another, costs of horn expression provide a plausible link between weapon diversity and ecology (Emlen 2001).

Here, the diversity of ecological habitats occupied by these beetles may matter. Beetles in all of these varied environments are likely to need to fly, smell, and see. Indeed, males must do all of these before they ever get a chance to use their horns (they cannot defend tunnels containing females unless they first find the dung and the females). However, the relative importance of these locomotory and sensory modalities may vary greatly depending on the ecological circumstances encountered by each beetle species, and this could affect the costs associated with each type of horn.

If the relative costs of each of the horns differ across selective regimes or habitats, then selection to minimize the functional costs of horn expression could lead to diversity in horn morphology. As beetles colonized new food sources or invaded new and different habitats, the relative costs of the different horn types may have shifted. In these populations, individuals producing horns that performed well *but cost less* in the new habitat may have replaced those that produced the older, more expensive, weapon forms, leading to diversification in the physical locations of horns. By mapping ecological characters on to the phylogeny, we provide three preliminary tests of this mechanism of divergence, involving each of the proposed trade-offs: horns versus wings, horns versus antennae, and horns versus eyes.

Beetles use their wings as they fly from breeding event to breeding event (e.g., sequential dung pads). The typical distance beetles need to fly between these breeding events could

alter the relative costs of horns on the thorax (H4, H5), if these horns stunt the growth of wings (Emlen 2001). Of the ecological characters we were able to measure, population density was the most likely to affect dispersal distance. Specifically, individuals in high-density (i.e., abundant) populations may need to travel much shorter distances, on average, than individuals in species characterized by lower population densities, and horns on the thorax may be least costly to these high-density species. In this case, if trade-offs are influencing patterns of horn diversification, then gains of thoracic horns (H4, H5) should be more likely in lineages with high population densities than in those with lower population densities.

Seven of the 11 gains of thoracic horns occurred in lineages characterized by extraordinarily high population densities (concentrated changes test: $P = 0.003$; Table 2). In contrast, gains of head horns, which are not predicted to trade off with wings, were not associated with population density (one of five gains of head horns, $P = 0.762$; Table 2). We suggest that while high population densities may have favored increased investment into male weaponry in general, the nature of this investment (i.e., which horn types were added) may be best explained by the relative costs of these horns in their respective environments.

Similarly, beetles use their antennae to detect odor plumes from dung, and odor plumes persist longer in forest understory habitats than they do in windier, more open pastures and fields (D. J. Emlen, unpubl. data). We predicted that if dung odors are especially difficult to detect in open habitats, then horns on the head (H2, H3, and to a lesser extent, H1) might be prohibitively costly in these environments, as their development stunts the relative growth of antennae (Emlen 2001). If trade-offs have influenced patterns of horn evolution, then we would expect gains of head horns to have occurred preferentially in forests, rather than in pastures. Four of the five gains of head horns occurred in forest habitats (concentrated changes test: $P = 0.059$, Table 2). Gains of thoracic horns (which are not predicted to affect antenna size) were not associated with forest versus pasture habitat preferences (three of 11 gains, $P = 0.921$).

The best evidence that costs may have influenced the evolution of horn morphology involves the ancestral horn type, horns at the base of the head (H1). Growth of this horn reduces the relative size of eyes by over 30% (Nijhout and Emlen 1998; Emlen 2000, 2001). Eyes are used for balance and orientation during flight (Gokan and Meyer-Rochow 1990), and one widespread pattern in dung beetles is that nocturnal species have disproportionately large eyes, presumably to see under low-light and/or crepuscular conditions (Caveney and McIntyre 1981; Krikken and Huijbregts 1987; McIntyre and Caveney 1998; for a similar pattern in bees see Jander and Jander 2002). For this reason, we predicted that horns at the base of the head might be especially costly to species that fly at night.

Because this horn type was already present in the majority of sampled taxa, we focused on losses of this horn, rather than gains. We predicted that if trade-offs have influenced the evolutionary diversification of horns, then losses of head horns (H1) should be most likely in lineages that have switched from diurnal to nocturnal flight behavior. Of the nine observed losses of H1, seven occurred on branches with nocturnal flight (concentrated changes test: $P = 0.000$).

Consequently, results of this study begin to address the question of why horn morphologies have changed so dramatically in form. At least part of this diversity appears to have resulted from selection to minimize the relative costs of horn growth. Existing horns were lost primarily in those habitats in which they were most costly. Where novel horns were gained, more often than not, the type of horn gained was the type predicted to be least expensive in that environment.

Additional studies will be needed to better discern which combinations (if any) of the four proposed mechanisms best explain the evolutionary radiation of beetle horns (Table 3). We end by briefly discussing the surprising losses of all horns and the equally surprising gains of horns in females.

Loss of Horns

Sexual selection is expected to continuously favor large weapon sizes (Parker 1979, 1983; West Eberhard 1983; Andersson 1994), and horned beetles fit this pattern: in every species tested thus far, males with the longest horns won disproportionate access to females (Eberhard 1982; Goldsmith 1987; Siva-Jothy 1987; Zeh and Zeh 1992; Rasmussen 1994; Emlen 1997; Moczek and Emlen 2000). Yet, our phylogeny suggests that complete loss of all horns has occurred at least three times (branches leading to *O. pennsylvannicus*; to *O. cochisus*, *O. coscineus*, and *O. knulli*; and to *O. muticus*). These losses remind us that the intensity (and even the direction) of sexual selection need not be constant over time, and that the costs of ornament or weapon production may, in some circumstances, become sufficiently prohibitive as to favor loss of trait expression (Wiens 2001). In dung beetles, several plausible circumstances could cause taxa with long histories of sexual selection for large horns to find themselves in ecological situations in which the costs became prohibitive. For example, extremely high population densities might make tunnel guarding impractical. Extremely low densities might render tunnel guarding unnecessary or favor females dispensing with tunneling altogether. Either of these situations could undermine the reproductive benefits of horns and, combined with the extensive costs of horn production, favor animals without horns. However, neither of these circumstances was correlated with losses of horns in our study (Table 3), and field studies of secondarily hornless species will be needed to better determine why horn expression may have been lost.

Evolution of Horns in Female Beetles

Female weapons are rare in animals (Darwin 1871; Andersson 1994). In general, males compete with other males over reproductive access to females, instead of the reverse situation (Darwin 1871; Trivers 1972; Thornhill and Alcock 1983; Andersson 1994), and most exaggerated animal weapons are used in these intrasexual contests over reproduction. However, there are some ecological situations where fights over food resources are especially intense, and it is possible that these situations favor the expression of weapons in females as well as males. Caribou depend on unusually limiting and patchy winter food resources, and competition for these

winter resources is thought to have led to female production of large antlers (Espmark 1964; Barrette and Vandal 1986; Lincoln 1994). Indeed, this species is unusual among the ungulates because females produce antlers comparable in size to those of the males.

Animal carcasses also comprise nutrient-rich resources that are patchily distributed and very rare (Scott 1990; Hanski and Cambefort 1991; Eggert and Müller 1992), and female insects regularly fight with other females for this resource (Pukowski 1933; Wilson and Fudge 1984; Otronen 1988; Müller et al. 1990; Scott 1990; Trumbo 1990; Eggert and Müller 1992). Carrion beetles (Coleoptera: Silphidae) do not produce horns in either males or females, but the beetle *Coprophanaeous ensifer* does. *Coprophanaeous ensifer* is a tunneling dung beetle (Coleoptera: Scarabaeidae: Phanaeini) typical of most species in its group (and similar to species of *Onthophagus*) because males bear large horns that are used in contests with rival males over tunnels containing females (e.g., *Phanaeus difformis* in Rasmussen 1994). However, *C. ensifer* is extraordinary in two ways: it feeds on animal carcasses instead of dung (Otronen 1988), and females also produce horns (Otronen 1988). Females of this species regularly fight with other females over possession of animal carcasses (Otronen 1988), and it may be that this unusually intense female-female competition favored females that coopted patterns of horn expression already present in the males.

Could similar situations explain the evolution of female horns in *Onthophagus*? If this were the case, then we would predict that species bearing female horns would also colonize types of dung that were especially sparsely distributed or ephemeral. In our study, the closest approximations to sparse/ephemeral food resources were low population density and diet specialization, and neither of these was correlated with gains of female horns (Table 2). If anything, the reverse was true: seven of the 13 gains of female horns occurred in species with high population densities (Table 2), suggesting that for these animals, large population sizes may generate the highest levels of female aggression.

An alternative possibility is that males and females assess each other as potential mates using fights (Otronen 1988). Dung beetles are notorious for extensive parental provisioning of young, and in many species this involves males as well as females (Fabre 1899; Halffter and Edmonds 1982; Sowig 1996; Hunt and Simmons 1998, 2000; Moczek 1998). In species with extensive biparental care, male investment can rival that of the females and these males often preferentially pair with the largest females (Otronen 1988; Scott 1990; Eggert and Müller 1992), or they provision most intensively when they are paired with the largest females (Cook 1988; Hunt and Simmons 2000; Hunt et al. 2002). If males in these species assess female size during contests, then females with horns might be favored more often than females without horns. In this case, we would predict that female horns would be most likely in species where males invest the most in parental care, where males fight with females, and where males pair or invest parental effort preferentially with the largest females.

It is even possible that extreme cases of male parental investment result in true role reversals, such that males now become the limiting sex and females compete with females over reproductive access to males. Females do appear to use their horns in contests with rival females (J. Marangelo, J. Hunt, D. Emlen, and L. Simmons, pers. obs.). However, preliminary observations of *O. sagittarius* suggest that these female contests arise over ownership of the tunnels themselves, rather than over males or stored food provisions (e.g., intruder females that successfully replace a resident female remove all of the dung provisioned by the first female before they begin to provision the tunnel themselves; J. Marangelo, unpubl. data). It is not yet clear why tunnels might be especially limiting in this species; nor is it clear whether these observations apply to the other species with female horns. We hope to address this in future papers.

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APPENDIX 1

Horn morphologies of species included in this study. Horns were scored as arising from one of five physical locations on the animals: head horns extended from the vertex (1), the frons (2), or the clypeus (3), and thoracic horns extended from the center (4) or sides (5) of the pronotum. Horns were scored separately for males and females of each beetle species. X indicates an enlarged weapon, (x) refers to the presence of rudimentary bumps (see text for explanation). Geographic regions reflect native locales (ignoring recent range expansions due to human translocation). Species labeled with an asterisk were determined to be near the named taxon.

Onthophagus species	Male horns					Female horns						References
	1	2	3	4	5	1	2	3	4	5		
acuminatus Harold	X										C. America	3, 6, 17, 18
aeruginosus Roth	X										Africa	22
alcyonides d'Orbigny	X		(x)								Africa	22, 23
asperulus d'Orbigny	X								(x)		Africa	22
australis Guerin	X								(x)		Australia	21
binodis Thunberg			(x)						(x)		Africa	22, 26, 27
capella Kirby	X		X	(x)				(x)	X		Australia	4, 11, 19, 21, 30
clypeatus Blanchard*	X		X	X					X		S. America	6, 24 (Onth. sp. 1)
cochisus Brown											N. America	15
coscineus Bates											C. America	3, 6, 17, 18
cribripennis d'Orbigny	X										Africa	22
crinitus panamensis Bates	X		(x)	(x)					(x)		C. America	3, 6, 17, 18
evanidus Harold	X										Australia	21
ferox Harold	X			X		X			X		Australia	4, 11, 19, 21, 26
fuliginosus Erichson	X										Australia	21
gazella Fabricius											Africa	13, 22, 30
granulatus Boheman			(x)	(x)	(x)				(x)	(x)	Australia	4, 11, 21, 30
haagi Harold		X		X			X		X		Australia	4, 11, 19, 21, 26
haemotopus Harold	X										S. America	6
hecate Panzer	(x)		(x)	X					(x)		N. America	5, 6, 8, 12, 14, 15, 20, 25, 27–29
incensus Say	X			(x)		(x)			(x)		C. America	3, 6, 15, 17, 18
knulli Howden & Cartwright											N. America	15
laminatus Macleay	X			X	(x)	X			X	(x)	Australia	4, 19, 21
lanista Castelnau	X			X		X			X		Africa	22
marginicollis Harold	X			(x)	(x)				(x)	(x)	C. America	3, 6, 17, 18
mjobergi Gillet	(x)			X	(x)	(x)			X	(x)	Australia	21
muticus Macleay											Australia	21
nigriventris d'Orbigny				X					X		Africa	22, 30
nuchicornis Linnaeus	X										Eurasia	6, 15, 28
orpheus Panzer	(x)			X					(x)		N. America	5, 6, 8, 12, 15, 25, 28
pennsylvanicus Harold											N. America	5, 6, 8, 12, 14, 15, 25, 28
pentacanthus Harold	X		X	X	X				X	X	Australia	4, 11, 19, 21, 30
praecellens Bates	(x)		X	X					X	X	C. America	3, 6, 17, 18
raffrayi Harold	X										Africa	22
rangifer Klug	X	X		(x)			X		(x)		Africa	1, 7, 22
sagittarius Fabricius											Eurasia	1
sharpi Harold	X		X	X		X			X		C. America	3, 6, 17, 18
sloanei Blackburn	(x)			X	(x)				X	(x)	Australia	4, 19, 21
stockwelli Howden & Young	X			(x)					(x)		C. America	17, 18
sugillatus Klug*	X										Africa	22
taurus Schreber	X		(x)	(x)	X	(x)			(x)	(x)	Eurasia	1, 2, 9, 10, 26, 30
tersidorsis d'Orbigny	X								(x)		Africa	22
vermiculatus Frey	X			(x)					(x)		Australia	21
xanthomerus Bates*	X		X	X					X		S. America	3, 6, 16, 24 (Onth. sp. 3), 31
Unknown sp. 1	X										S. America	
Unknown sp. 2	X										S. America	
Unknown sp. 3	X										S. America	
Unknown sp. 4	X										Africa	

(1) Arrow 1951; (2) Balthasar 1963; (3) Bates 1886–1890; (4) Blackburn 1903; (5) Blatchley 1910; (6) Boucomont 1932; (7) Darwin 1871; (8) Dawson 1924; (9) Fabre 1899; (10) Fincher and Woodruff 1975; (11) von Harold 1869; (12) Helgesen and Post 1967; (13) Hoebeke and Beucke 1997; (14) Horn 1875; (15) Howden and Cartwright 1963; (16) Howden and Nealis 1975; (17) Howden and Young 1981; (18) Kohlmann and Solís 2001; (19) Lea 1923; (20) Lindquist 1933; (21) Matthews 1972; (22) d'Orbigny 1913; (23) Palestrini and Giacone 1989; (24) Peck and Forsyth 1982; (25) Ratcliffe 1991; (26) Risdill-Smith et al. 1989; (27) Ritcher 1945; (28) Schaeffer 1914; (29) Sturm 1843; (30) Tyndale-Biscoe 1990; (31) Zunino 1981.

APPENDIX 2

Scoring of ecological characters and gene sequences included in this study. N, nocturnal (flies at night); F, forest inhabitant; S, diet specialist; A, abundant; R, rare.

Onthophagus species	Ecological character					Gene						
	N	F	S	A	R	16s	CO1	CO2	28s	3059	3089	8029
acuminatus Harold	no	yes	no	yes	no	X	X	X	X		X	X
aeruginosus Roth	no	no	no	no	yes	X	X	X	X	X	X	X
alcyonides d'Orbigny	no	no	yes[2]	no	yes	X	X	X	X	X		
asperulus d'Orbigny	no	no	no	no	no	X	X	X	X	X	X	X
australis Guerin	no	no	no	no	no	X	X	X	X			
binodis Thunberg	yes[1]	no	no	yes	no	X	X		X	X		
capella Kirby	yes	no	no	no	no	X	X	X	X			
clypeatus Blanchard*	no	yes	no	?	?	X	X	X	X			
cochisus Brown	yes	yes	no	no	yes	X	X	X	X			
coscineus Bates	yes[1]	yes	no	no	no	X	X	X	X	X	X	X
cribripennis d'Orbigny	no	no	no	no	yes	X	X	X	X	X	X	X
crinitus panamensis Bates	no	yes	no	no	no	X	X	X	X			
evanidus Harold	no	yes	no	no	no		X		X	X		
ferox Harold	yes	no	no	yes	no	X	X		X	X		X
fuliginosus Erichson	no	yes	no	no	no	X			X	X		
gazella Fabricius	yes[1]	no	no	yes	no	X	X	X	X		X	X
granulatus Boheman	no	no	no	yes	no	X	X	X	X	X		X
haagi Harold	no	yes	yes[3]	no	yes	X	X		X	X		X
haemotopus Harold	no	yes	no	no	no	X	X	X	X			
hecate Panzer	yes[1]	no	no	yes	no	X	X	X	X	X	X	X
incensus Say	no	no	no	yes	no	X	X	X	X	X	X	X
knulli Howden & Cartwright	yes	no	no	no	no	X	X		X			
laminatus Macleay	yes	no	no	no	yes	X	X	X	X			
lanista Castelnau	no	no	no	yes	no	X			X			
marginicollis Harold	no	no	no	yes	no	X	X	X	X	X		
mjobergi Gillet	yes	no	no	no	yes	X	X	X	X	X	X	X
muticus Macleay	yes	no	yes[4]	no	yes	X						
nigriventris d'Orbigny	no	no	no	yes	no	X	X	X	X			
nuchicornis Linnaeus	no	no	no	no	no	X	X	X	X	X	X	X
orpheus Panzer	yes[1]	yes	yes[5]	no	yes	X	X	X	X			
pennsylvanicus Harold	yes[1]	no	no	yes	no	X	X	X	X	X	X	X
pentacanthus Harold	yes	no	no	no	no	X	X	X	X			
praecellens Bates	yes[1]	yes	no	no	no	X	X	X	X			
raffrayi Harold	no	no	no	yes	no	X			X			
rangifer Klug	no	no	no	no	no	X	X		X			
sagittarius Fabricius	yes[1]	no	no	yes	no	X	X	X	X	X	X	X
sharpi Harold	yes	yes	yes[6]	no	no	X	X	X	X			
sloanei Blackburn	yes	?	?	no	yes	X	X	X	X			
stockwelli Howden & Young	no	yes	no	no	yes		X	X	X			
sugillatus Klug*	no	no	no	no	yes	X	X	X	X	X	X	X
taurus Schreber	no	no	no	yes	no	X	X	X	X	X	X	X
tersidorsis d'Orbigny	no	?	?	yes	no	X		X	X	X		
vermiculatus Frey	no	yes	no	no	no	X	X		X	X	X	X
xanthomerus Bates*	no	yes	no	?	?	X	X	X	X			
Unknown sp. 1	no	yes	?	?	?	X	X	X	X			
Unknown sp. 2	?	yes	?	?	?	X	X		X			
Unknown sp. 3	no	yes	no	yes	no	X		X	X			
Unknown sp. 4	no	no	?	no	yes	X	X		X			

[1] Flies in the daytime as well as at night.
[2] Kleptoparasite of other dung beetles.
[3] Feeds on carrion.
[4] Clings to the fur of marsupials.
[5] Lives in mammal burrows (e.g., woodchuck, woodrat).
[6] Feeds on rotting fruit.