ARBOREALITY AND MORPHOLOGICAL EVOLUTION IN GROUND BEETLES (CARABIDAE: HARPALINAE): TESTING THE TAXON PULSE MODEL

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Abstract.—One-third to two-thirds of all tropical carabids, or ground beetles, are arboreal, and evolution of arboreality has been proposed to be a dead end in this group. Many arboreal carabids have unusual morphological features that have been proposed to be adaptations for life on vegetation, including large, hemispheric eyes; an elongated prothorax; long elytra; long legs; bilobed fourth tarsomeres; adhesive setae on tarsi; and pectinate claws. However, correlations between these features and arboreality have not been rigorously tested previously. I examined the evolution of arboreality and morphological features often associated with this habitat in a phylogenetic context. The number and rates of origins and losses of arboreality in carabids in the subfamily Harpalinae were inferred with parsimony and maximum-likelihood on a variety of phylogenetic hypotheses. Correlated evolution in arboreality and morphological characters was tested with concentrated changes tests, maximum-likelihood, and independent contrasts on optimal phylogenies. There is strong evidence that both arboreality and the morphological features examined originated multiple times and can be reversed, and in no case could the hypothesis of equal rates of gains and losses be rejected. Several features are associated with arboreality: adhesive setae on the tarsi, bilobed tarsomeres, and possibly pectinate claws and an elongated prothorax. Bulgy eyes, long legs, and long elytra were not correlated with arboreality and are probably not arboreal adaptations. The evolution of arboreal carabids has not been unidirectional. These beetles have experienced multiple gains and losses of arboreality and the morphological characters commonly associated with the arboreal habitat. The evolutionary process of unidirectional character change may not be as widespread as previously thought and reversal from specialized lifestyles or habitats may be common.

Key words.—Ancestral state reconstruction, correlated character evolution, habitat specialization, independent contrasts, maximum-likelihood, niche shift, phylogenetic uncertainty.

Ecological niche shifts may be key in generating patterns of organismal diversity because a niche shift will generally lead to decreased competition and permit the performance of new functions that will open new adaptive zones (Simpson 1953). Increasing taxonomic diversity and progressive specialization within a lineage allow the exploitation of new habitats. Adaptations acquired during such a radiation possess both an ecological function and a unique evolutionary history. For carabids, commonly known as ground beetles, vegetation can be considered a new adaptive zone, invasion of which might promote radiation and diversification in some groups. Carabids are ancestrally ground-dwelling (Erwin 1979, 1994; Baehr 1998), and one model of evolution for the group suggests that vegetation is a new niche for these beetles. Once invaded, their descendants cannot revert to the ancestral habitat.

Understanding the evolution of shifts to new niches is one of the major unresolved issues in evolutionary biology (Mayr 1942; Simpson 1944; Fox and Morrow 1981; Bernays and Graham 1988; Futuyma and Moreno 1988; Jaenike 1990; Kelley and Farrell 1998). Phylogenetic evidence for resource shifts is not common, however, and few phylogenetic studies directly test for the evolutionary rates and direction of niche shifts (Futuyma and Moreno 1988; Farrell and Mitter 1993; Thompson 1994; Schluter 2000; Nosil 2002). Using phylogenetic tools, I tested the validity of this model of niche shift and its underlying assumptions, specifically, the common assumption that evolutionary trends expressed during radiation and diversification are irreversible.

Ecological resource niche shifts are often argued to be irreversible, or more likely to change in one direction than the reverse (Mayr 1942; Simpson 1953; Bull and Charnov 1985). However, empirical studies have challenged many of the assertions regarding absolute or partial irreversibility (see Teotónio and Rose 2001). A well-known, but relatively poorly tested, principle in biology, Cope's rule, involves evolutionary irreversibility. Cope (1896) suggested in his ''law of the unspecialized'' that successful, species-rich lineages usually originated from generalized ancestors with developmental and ecological flexibility, not restricted to definite habitats, climate or other resources. In contrast, specialized forms were evolutionary ''dead ends'' and became extinct when faced with major environmental changes (Stanley 1973; Hayami 1978). Suites of adaptations and an increasing commitment to exploiting a specific new resource may constrain the ability to revert to a previous habit in the future (Schluter 2000). Cope (1896) did not specify a mechanism for the restricted evolutionary process; however, limitations on reversals could result from the loss of complex traits. Reversal may be improbable because evolutionary adaptation to new niches or other complex traits involves epistatic interactions that cannot be lost or easily dismantled (see Bull and Charnov 1985; Teotónio and Rose 2001). Strong directional or stabilizing selection favoring new habitat specialization, disadvantageous intermediates, or lack of genetic variation can lead lineages into evolutionary blind alleys. Patterns of irreversible evolution into new niches or new modes of life have been observed in *Anolis* lizards (Losos 1992; Losos et al. 1994), land snails (Vermeij 2000), and ectomycorrhizal fungi (Cullings et al. 1996).

Erwin (1979, 1985) proposed the taxon pulse model of unidirectional evolution, in which arboreal carabid beetles

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evolved from terrestrial lineages, but not the reverse, thereby providing a potential example of Cope's rule. The radiation of arboreal and terrestrial carabid beetles provides an opportunity to investigate the directional changes in ecological niches. Has the shift from terrestrial to arboreal habitats been unidirectional in carabids, or have reversals from arboreality back to ground-dwelling taken place in the evolutionary history of these beetles? How many times have habitat shifts occurred? Morphological features are often associated with entry into a new set of ecological niches; therefore, I also investigate the evolution of morphological characters that have been hypothesized to coevolve with arboreality in carabids.

Arboreal Carabids

Carabids are one of the largest groups of terrestrial predators and one of the largest families of beetles. Arboreal carabids are especially diverse in tropical regions, where the arboreal carabid community comprises nearly two-thirds of all tropical, lowland carabids (Darlington 1971; Erwin 1979; Stork 1987). Most carabids (including most arboreal forms) are placed in the subfamily Harpalinae. Harpalines are diverse in morphological forms and ecological interactions (Erwin 1979). Diversity within harpalines, including arboreal forms, may have arisen through a series of unidirectional adaptive shifts and subsequent radiations, known as taxon pulses, from one habitat to another (Erwin 1979, 1985). According to this model, waves of lineages began as streamside generalist predators in tropical lowlands, dispersed out from that habitat to the forest floor, and moved into rainforest canopies (and other kinds of isolated ecological islands such as mountaintops and caves). The taxon pulse model suggests that these adaptive shifts lead to isolation of older lineages of carabids in marginal or specialized habitats and eventually extinction (Erwin 1979, 1985, 1998).

Carabid beetles are ancestrally ground-dwelling, probably associated with wet habitats (Erwin 1979; Beutel and Haas 1996; Maddison et al. 1999; Shull et al. 2001). Outside of harpalines, there are few arboreal carabids (Erwin 1994; Baehr 1998), and they are deeply nested within ancestrally terrestrial clades (Maddison et al. 1999; D. Maddison and K. Ober, unpubl. data). The ancestor of Harpalinae plus its close relatives, austral psydrines and brachinines (Ober 2002) was ground-dwelling; as a result arboreality within harpalines represents one or more invasions of a new niche for carabids.

Many harpalines are adapted to an exclusively arboreal life in the tropical rainforest canopy (e.g., *Agra*, Erwin and Pogue 1988), whereas others spend most of their time on the ground, but occasionally hunt for insect prey on small shrubs or grasses (e.g., *Calathus*, pers. obs.), or climb plants to feed on seeds (e.g., *Amara*, Lindroth 1968; Thiele 1977; Forsythe 1982). For carabids, life on plants is different from life on the ground. Arboreal carabids live in a generally more exposed environment and must deal with such problems as holding onto plant surfaces, finding food and oviposition sites on vegetation, and escaping predators in arboreal habitats (Stork 1987).

Adaptive value has been proposed for a variety of morphological traits found in arboreal carabids. Carabids that live under bark or on leaves, twigs, trunks of trees, and small shrubs often have distinct features not found in terrestrial groups. For instance, bilobed or widely expanded tarsomeres may be an adaptation for walking on leaves (Habu 1967; Erwin 1979, 1985; Erwin and Pogue 1988). Long, thin legs may help carabids climb narrow plant stems (Habu 1967) or gain a wide stance when holding on to leaves or tree trunks (Erwin 1979). Erwin (1979) and Erwin and Pogue (1988) also suggested that carabids feeding on insects on tree trunks have long narrow legs for high-speed running and for elevating themselves for better vision in a three-dimensional habitat. Arboreal carabids commonly possess pectinate claws. The teeth on the claws of arboreal carabids may assist in gripping bark and leaf surfaces by catching against trichomes or scales, or even in open stomata (Stork 1987). The legs have numerous large brushes of subtarsal adhesive setae that are long and curved or spatulate (Stork 1980; Erwin 1979) and are clustered together in large brushes on all legs. Carabids with an elongate and relatively narrow body shape are commonly found in arboreal habitats. Most elongate arboreal forms are highly active leaf or bark runners (Erwin 1979). Elongation may have to do with pupation site (e.g., Ctenodactylini inside grass stems) or hiding or hunting in crevices of bark, leaf axils, or burrows in wood (Erwin 1979; Erwin and Pogue 1988). Bright metallic colors for foliage dwellers, dull colors for bark-runners, and large hemispheric eyes have been proposed to be adaptations to life on vegetation (Erwin 1979). While many studies have been done in arboreal vertebrates examining the functional performance of arboreal adaptations such as limb length and body shape (Prost and Sussman 1969; Cartmill 1985; Higham et al. 2001), to date, neither functional nor phylogenetic tests have been done for the adaptive value of any of these traits in arboreal carabids.

This paper examines the evolutionary shifts of carabids between terrestrial habitats and arboreal habitats and the morphological characters associated with arboreality in the context of a phylogenetic hypothesis. To investigate the evolution of arboreality in Harpalinae, it is necessary to account for the phylogenetic relationships within the subfamily and to infer the rates and likely pattern of origins and losses of arboreality. This study explores the evolution of arboreality in harpalines by examining a variety of phylogenies estimated from different methods (parsimony, minimum evolution distance, and Bayesian) to account for phylogenetic uncertainty and seeks to determine the number and rate of origins and losses of arboreality. I also explore, in a phylogenetic framework, the correlated evolution of arboreality with morphological characters such as an elongated prothorax; large, hemispheric eyes; long legs; pectinate claws; and expanded tarsi with adhesive setae. These morphological characters are suggested to be arboreal adaptations for locomotion, prey capture, or avoiding predators. The main questions addressed in this study are: (1) How many origins and losses of arboreality have there been within harpalines? (2) Are the rates of origin and loss of arboreality equal? (3) Have reversals to ground-dwelling occurred? (4) Are there morphological characters such as an elongated prothorax; large, hemispheric eyes; long legs; pectinate claws; and expanded tarsi with adhesive setae, correlated with an arboreal lifestyle?

METHODS

Taxon Sampling

Morphological data were gathered from 443 specimens representing all 186 harpaline species (see Electronic Appendix currently available from the *Evolution* Editorial Office at evolution@asu.edu) that were included in the molecular phylogenies of Ober and Maddison (2001). An effort was made to include males and females. Outgroups to Harpalinae were included in the molecular phylogenetic inferences (Ober and Maddison 2001) and were used to root the harpaline phylogeny, but were not examined in the analyses of arboreality and morphological character evolution. Taxa were chosen to represent the greatest diversity within tribes from material to which I had access. Within a genus, little variation exists in most morphological features and in habitat preference (Erwin 1979). Many tribes are either all arboreal or all terrestrial (Erwin 1979). In some tribes, however, both habitat preferences are observed (e.g., Pentagonicini, Lebiini, and Cyclosomini). In those tribes, I sampled based mainly on classification, and I included representatives of a diversity of both terrestrial and arboreal genera.

Phylogenetic Hypotheses

One minimum evolution distance tree (Fig. 1) and 800 most parsimonious trees (MPTs) on two islands (Maddison 1991), inferred from the combined molecular sequence dataset of 28S rDNA and the *wingless* gene (Ober and Maddison 2001), were used as the hypotheses of phylogenetic relationships among tribes of Harpalinae. Although support for many of the clades in these trees was not strong (Fig. 1; Ober and Maddison 2001), they are the best current estimate of harpaline phylogeny.

To incorporate uncertainty about the true phylogeny, I examined additional trees from 100 parsimony and 100 neighbor-joining (HKY85 distance measure) bootstrap replicates (Ober and Maddison 2001). The trees from the bootstrap replicates were used as diverse alternative tree topologies inferred from the molecular data. If support for hypotheses about arboreality in harpalines is provided by character state reconstructions on all the alternative trees, then the conclusions based on these reconstructions are probably robust to errors in the phylogeny estimation (Swofford and Maddison 1992).

I performed a Bayesian phylogenetic analysis of the 28S + *wingless* combined dataset from Ober and Maddison (2001), using MrBayes 2.01 (Huelsenbeck and Ronquist 2001) to estimate the posterior probability of phylogenetic trees for harpalines and to better understand how phylogenetic uncertainty and differences in tree topology can influence the reconstruction of ancestral states when examining the origin and losses of traits. The Markov chain Monte Carlo (MCMC) procedure ensures that trees are sampled in proportion to their posterior probability under the model of gene sequence evolution (Huelsenbeck and Ronquist 2001). One cold and three heated MCMC chains were run for 400,000 generations sampling every 10th tree. Harpalines were constrained to be monophyletic (Ober and Maddison 2001; Ober 2002). A GTR + Γ model of sequence evolution was used

to calculate the lnL of each tree and the prior probability for all trees was equal. Convergence of the Markov chains, or "burn-in," was evident after 180,000 generations. The first 18,000 trees were discarded, and 22,000 trees were saved for character evolution analyses of arboreality. Figure 2 shows a tree of all nodes with greater than 0.5 posterior probability.
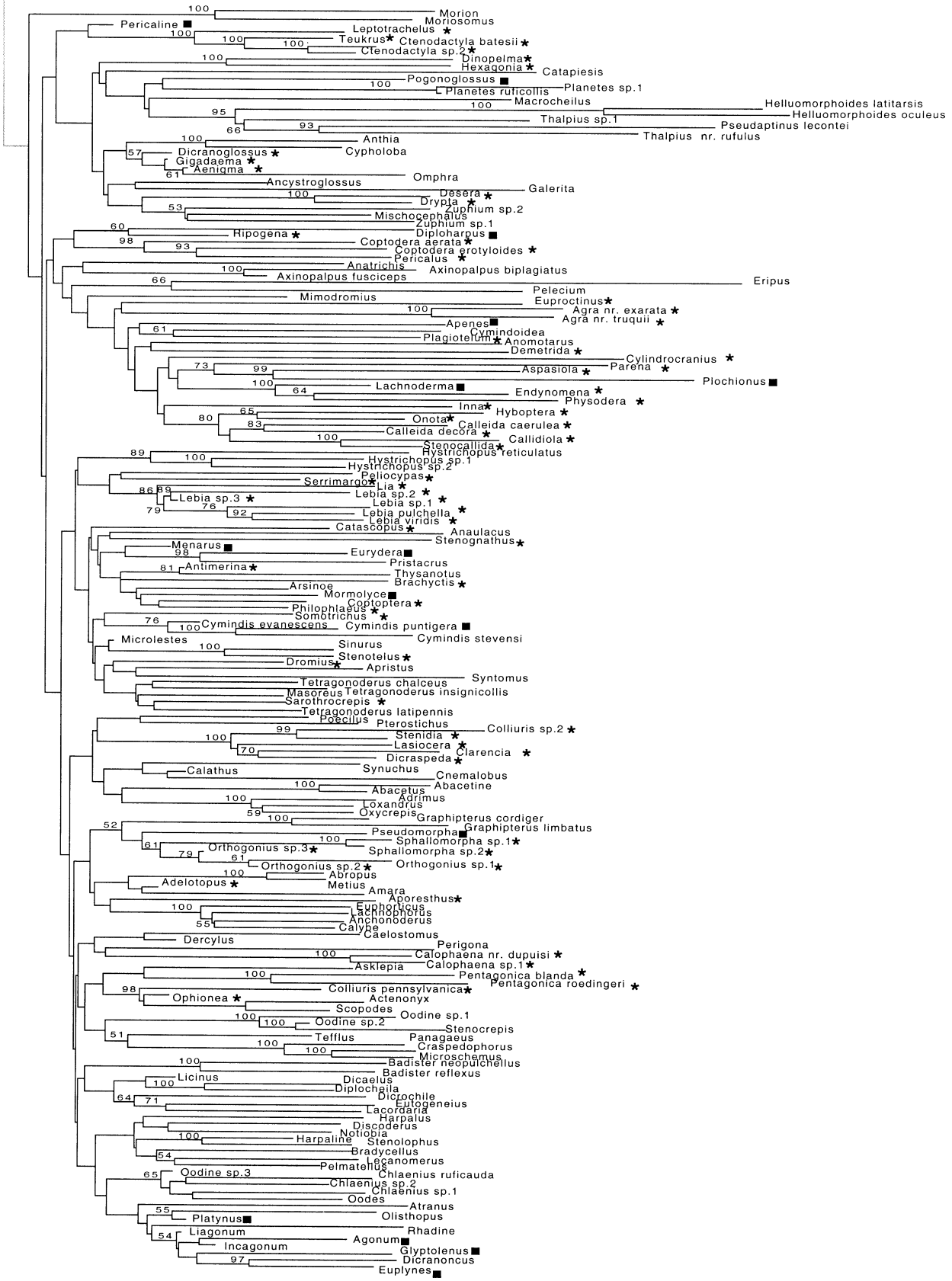
Morphological and Habitat Data

Ten measurements of adults were made using a digital camera on a stereoscopic dissecting microscope: HL: length of head along midline, from apex of clypeus to posterior margin of eye; EyW: width of head from outer edges of eye; NW: width of neck constriction measured at the occiput behind eyes; PL: length of pronotum along midline; PW: maximum width of pronotum; EL: length of elytra from apex of scutellum to elytral apex, including any apical points; ElW: maximum width of elytra; FL: maximum length of femur of last leg; TiL: maximum length of tibia of last leg; and TaL: maximum length of tarsus of last leg, excluding claws. The number of pixels for each measurement was recorded with NIH Image 1.62 and converted to millimeter with a digital image of a micrometer at the same magnification. Measurements were averaged over all specimens for a species and averaged for each sex separately.

EyW/NW approximated the bulginess of the eyes, a measure of how hemispheric the eyes are, or how far the compound eyes protrude from the head (Fig. 3). The prothorax shape was coded in two ways, a continuous character of the ratio of PL/PW and a binary character of the presence or absence of prothorax longer than wide (PL/PW > 1.0, Fig. 3). Elytral shape character was a ratio of EL/ElW. The relative leg length (TLL/TBL) was recorded as total leg length/total body length ([FL + TiL + TaL]/[HL + PL + EL]). I recorded the presence or absence of expanded or bilobed fourth tarsomeres, the presence or absence of adhesive setae on the underside of the fourth tarsomere of the last legs, and the number of teeth on the tarsal claws of the last legs (Fig. 3). Figure 3 shows some of the variation displayed by harpalines in bulginess of the eyes, shape of the prothorax, and shape of the fourth tarsomere of the legs. Leg characters were only recorded from the last legs to avoid secondary sexual characteristics. Many species of male carabids have special features for mating on their first, and sometimes second, pair of legs and can include expanded tarsomeres with adhesive setae to contact the female during copulation (West 1862; Jeannel 1941; Stork 1980).

Discrete characters did not vary by sex within a species for the specimens I examined, but relative leg length varied significantly between males and females (*t*-test, $P < 0.01$) in some species (e.g., *Harpalus caliginosus*, *Pterostichus melanarius*). Analyses of continuous characters were performed on the data collected from all specimens, from males only, and from females only.

Habitat data were collected from the literature, specimen labels, and information provided by carabid collectors (including my own fieldwork). Harpalines were scored as arboreal if a species spends most of the time on living vegetation, or terrestrial if it is ground-dwelling or lives in decaying wood. In this definition, arboreality is expanded to

Carabid outgroups

100 Morion
Moriosomus
Pericaline ■
Leptotrachelus ★
100 Teukrus ★ Ctenodactyla batesii ★
100 100 Ctenodactyla sp.2 ★
100 Dinopelma ★
Hexagonia ★ Catapiesis
100 Pogonoglossus ■
Planetes ruficollis Planetes sp.1
100 Macrocheilus
95 Helluomorphoides latitarsis
Helluomorphoides oculeus
66 93 Thalpius sp.1
Pseudaptinus lecontei
Thalpius nr. rufulus
100 Anthia
57 Dicranoglossus ★ Cypholoba
Gigadaema ★
61 Aenigma ★
Ancystroglossus Omphra
100 Desera ★ Galerita
Drypta ★
53 Zuphium sp.2
Mischocephalus
60 Zuphium sp.1
98 Hipogena ★ Diploharpus ■
93 Coptodera aerata ★
Coptodera erotyloides ★
Pericalus ★
100 Anatrichis Axinopalpus biplagiatus
66 Axinopalpus fusciceps Eripus
Pelecium
Euproctinus ★
Mimodromius 100 Agra nr. exarata ★
Agra nr. truquii ★
Apenes ■
61 Cymindoidea
Plagiotelum ★ Anomotarus
Demetrida ★
73 99 Cylindrocranius ★
Aspasiola ★ Parena ★
100 64 Plochionus ■
Lachnoderma ■ Endynomena ★
Inna ★ Hyboptera ★ Physodera ★
80 65 Onota ★ Calleida caerulea ★
83 Calleida decora ★ Callidiola ★
100 Stenocallida ★
Hystrichopus reticulatus
89 100 Hystrichopus sp.1
Hystrichopus sp.2
Peliocypas ★
Serrimargo ★ Lia ★
86 89 Lebia sp.2 ★
Lebia sp.3 ★ Lebia sp.1 ★
79 76 92 Lebia pulchella ★
Lebia viridis ★
Catascopus ★
Anaulacus
Stenognathus ★
Menarus ■
98 Eurydera ■
Pristacrus
81 Antimerina ★ Thysanotus
Brachyctis ★
Arsinoe Mormolyce ■
Coptoptera ★
Philophlaeus ★
Somotrichus ★
76 100 Cymindis evanescens Cymindis puntigera ■
Cymindis stevensi
Microlestes
100 Sinurus
Stenotelus ★
Dromius ★ Apristus
Syntomus
Tetragonoderus chalceus
Masoreus Tetragonoderus insignicollis
Sarothrocrepis ★
Tetragonoderus latipennis
Poecilus Pterostichus
99 Colliuris sp.2 ★
100 Stenidia ★
Lasiocera ★
70 Clarencia ★
Dicraspeda ★
Calathus Synuchus
Cnemalobus
100 Abacetus Abacetine
100 Adrimus
Loxandrus
59 100 Oxycrepis Graphipterus cordiger
Graphipterus limbatus
52 100 Pseudomorpha ■ Sphallomorpha sp.1 ★
Sphallomorpha sp.2 ★
61 Orthogonius sp.3 ★
79 61 Orthogonius sp.2 ★ Orthogonius sp.1 ★
100 Abropus
Metius
Adelotopus ★ Amara
Aporesthus ★
100 Euphorticus
Lachnophorus
55 Anchonoderus
Calybe
Dercylus Caelostomus
Perigona
100 Calophaena nr. dupuisi ★
Asklepia Calophaena sp.1 ★
100 Pentagonica blanda ★
98 Pentagonica roedingeri ★
Colliuris pennsylvanica ★
Ophionea ★ Actenonyx
Scopodes
100 Oodine sp.1
100 Oodine sp.2
Stenocrepis
51 Tefflus Panagaeus
100 100 Craspedophorus
Microschemus
Badister neopulchellus
100 Badister reflexus
100 Licinus Dicaelus
Diplochila
64 71 Dicrochile
Eutogeneius
Lacordaria
Harpalus
Discoderus
100 Notiobia
Harpaline Stenolophus
54 Bradycellus
Lecanomerus
Pelmatellus
65 Oodine sp.3 Chlaenius ruficauda
Chlaenius sp.2
Oodes Chlaenius sp.1
Atranus
55 Platynus ■ Olisthopus
Rhadine
54 Liagonum Agonum
Incagonum Glyptolenus ■
97 Dicranoncus
Euplynes ■

include species that live on or under bark of live trees and on herbaceous plants, as well as in the canopy of trees. Microhabitat information was also collected in a more detailed manner to explore the transitions between more finely parsed habitat types. Harpalines were coded as terrestrial, living in or on the ground; subcanopy dweller, living on forbs, grasses, shrubs, and occasionally found on the ground; canopy specialist, living most of its life on vegetation; dead wood dweller, living in dead logs or snags; or corticolous, living on or under the bark of living trees. Information on habitats was from adult beetles. Some unidentified species and species with conflicting reports about habitat were coded as uncertain or as missing data.

Character Evolution Analyses

To investigate character evolution of harpalines, I used a variety of methods of analysis and multiple phylogenetic trees inferred from molecular data. Parsimony, maximum-likelihood, and independent contrasts were used to examine the origins and losses of characters and correlations between arboreality and morphological traits on trees inferred from parsimony and distance methods.

The number of gains and losses in discrete characters (habitat, presence or absence of arboreality, prothorax shape, tarsomere shape, presence or absence of adhesive setae on tarsomeres, and claw structure) was analyzed on the MPTs and the distance tree with MacClade 4.01 (Maddison and Maddison 2001). Acquiring the states of arboreality, an elongated prothorax, expanded or bilobed tarsomeres, presence of adhesive setae on the tarsomeres, and pectinate claws will be referred to as ''gains'' or ''origins'' in this paper and an evolutionary change away from these states as a ''loss.'' These morphological character states have been associated with arboreality (Erwin 1979; Stork 1987). All of the most parsimonious reconstructions (MPRs) were examined for each character.

The gains and losses of arboreality were also reconstructed with parsimony on trees from 100 parsimony bootstrap replicates, trees from 100 neighbor-joining bootstrap replicates, and 22,000 trees from the Bayesian analysis of the molecular data. All MPRs of arboreality were examined for these trees. For the Bayesian analysis, the parsimony reconstruction of gains and losses of arboreality on each tree allowed the calculation of the number of gains and losses with the highest posterior probability (Huelsenbeck et al. 2000).

Rates of evolution of arboreality and discrete morphological characters (prothorax shape, tarsomere shape, presence or absence of adhesive setae, and claw structure) were estimated with a Markov model in a maximum-likelihood framework in DISCRETE (Pagel 1994). The parameters of trait evolution were estimated by summing the likelihood over all possible states at each node of the tree (Pagel 1994). The

states at the root of harpalines were not fixed, because the ancestral states of the subfamily were not known.

For each character, I examined the following two questions: Are the rates of origins and losses equal? Does a model with a loss (reversal) rate of zero fit the data significantly worse? Likelihood-ratio tests were performed by comparing the likelihood of a model where the rates of origins and losses of arboreality and the morphological characters were estimated from the data (i.e., free to vary) to the likelihood of a model where the gain and loss rates were constrained to be equal. Analyses were also done comparing the likelihood of a model where the loss or reversal rate of a character was estimated from the data to a model where the loss rate was constrained to be zero. The differences in the ln likelihoods were then compared with a likelihood-ratio test.

DISCRETE was also used to test for correlated evolution between arboreality and each of the discrete morphological characters. This was done by comparing the fit (likelihood) of two models to the data. In the first model the two characters are allowed to evolve independently (null hypothesis); in the second model they evolve in a correlated manner. Evidence for a correlation is found if the model of correlated evolution fits the data significantly better than the model of independent evolution with a likelihood-ratio test (Pagel 1994).

Character evolution in harpalines was explored with maximum-likelihood on the distance tree and one of the MPTs with branch lengths (constrained to be nonnegative) estimated from 28S rDNA by PAUP* version 4.0b8 (Swofford 2001). Branch lengths were estimated from 28S data alone because some taxa had missing sequence data from the *wingless* gene. Zero length branches were set to 10^{-8}, because DISCRETE cannot accept branch lengths smaller than or equal to zero. All tests were also done with zero length branches set to 10^{-5} and 10^{-10} to explore how these arbitrarily small branch lengths affected the likelihood estimations. In addition, tests were also performed in DISCRETE with all branch lengths of equal length and estimated by a scaling parameter kappa (κ) with maximum-likelihood (Pagel 1994). Taxa with polymorphic or uncertain character states for arboreality (15 taxa of 186) were set to one or zero for these tests based on the best evidence from the literature, the likelihoods were calculated, and the tests were performed again with the alternative character state for these taxa.

The concentrated changes test (Maddison 1990) was used to test whether gains in the discrete morphological characters (e.g., elongated prothorax, pectinate claws) occur more frequently in arboreal lineages than expected by chance. The null hypothesis is that changes in morphological characters are randomly distributed across terrestrial and arboreal lineages. One MPT chosen arbitrarily from each island of MPTs and the distance tree were used as phylogenetic hypotheses in the concentrated changes tests for arboreality and the mor-

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FIG. 1. Minimum evolution distance tree of the subfamily Harpalinae inferred from combined 28S rDNA + *wingless* dataset (Ober and Maddison 2001). Numbers on branches are bootstrap percentages. Taxa with an asterisk are arboreal. Taxa with a box are polymorphic or uncertain for habitat. Taxa without symbols are terrestrial. This tree along with parsimony trees, trees from neighbor joining and parsimony bootstrap replicates, and trees from Bayesian analysis were used to explore character evolution. Parsimony trees and details of the phylogenetic analyses can be found in Ober and Maddison (2001).
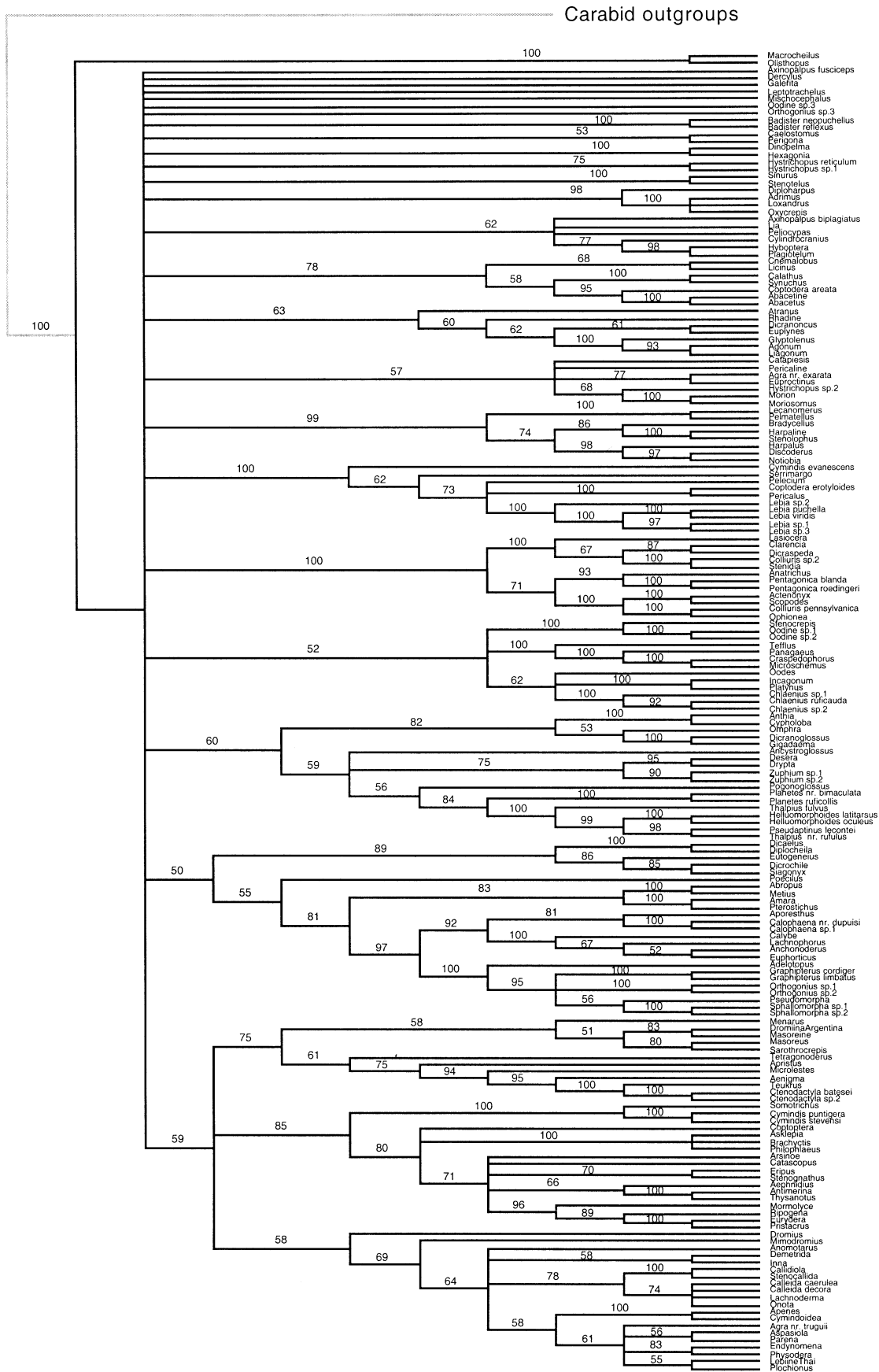
FIG. 2. Majority rule consensus tree of the 22,000 trees resulting from the Bayesian analysis of 28S rDNA + *wingless* combined dataset for taxa within the harpaline subfamily. Numbers above the branches indicate the probability of each clade.
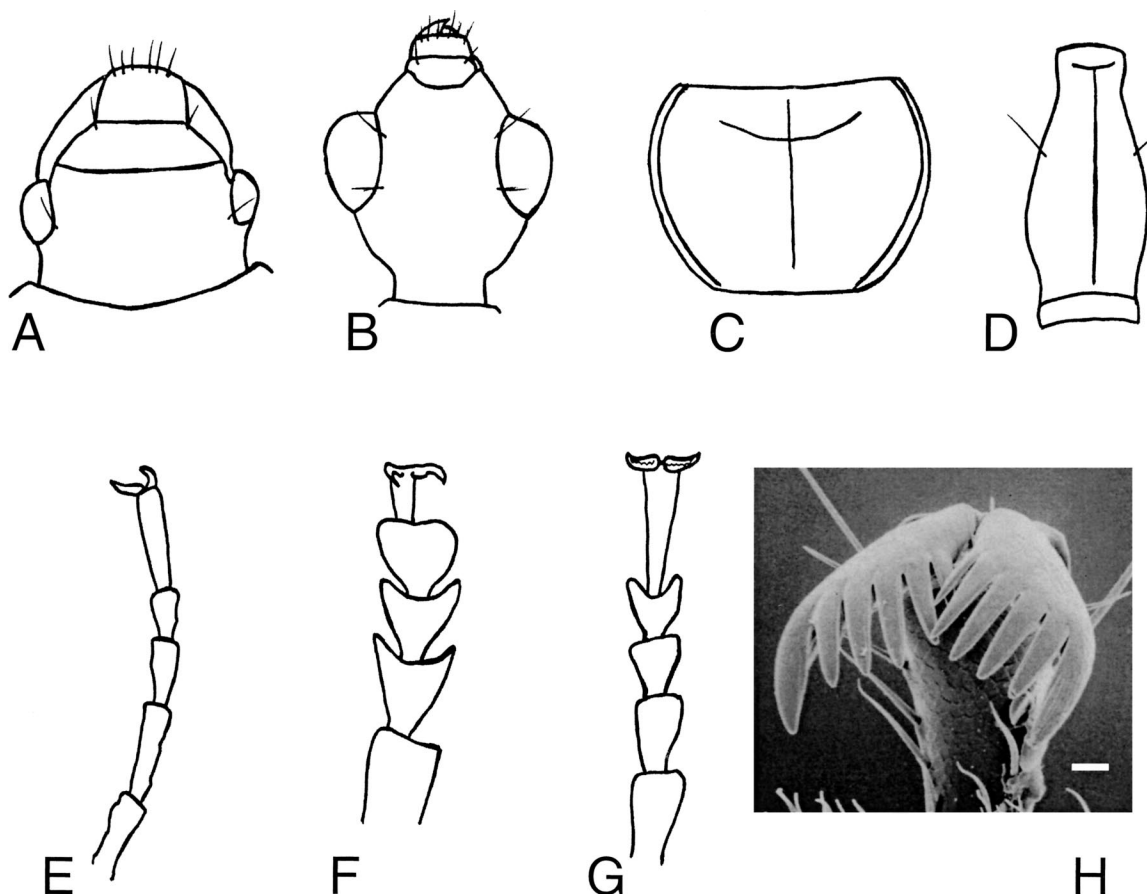
FIG. 3. Examples of morphological characters examined as adaptations for arboreality. Small eyes of *Harpalus caliginosus* (A), which spends most of its time on the ground, and the large, hemispheric eyes of *Calophaena* n. sp. (B), an arboreal harpaline. Wide pronotum of a terrestrial harpaline, *Discoderus cordicollis* (C), and narrow pronotum of *Ophionea ishii* (D), which lives on vegetation. Cylindrical fourth tarsomere of the leg of terrestrial *Lachnophorus elegantulus* (E), expanded fourth tarsomere of *Calophaena* n. sp. (F), and bilobed fourth tarsomere of arboreal *Lebia* sp. (G). Tarsal claw of an arboreal carabid, *Calleida decora* (H), showing the teeth of the claw; scale line = 16.2 μm. Illustrations not drawn at same scale.

phological characters. The tree topology differences within parsimony islands did not affect mapping of habitat or morphological characters, except for pectinate claws in parsimony island 2. In the latter case, I examined the evolution of pectinate claws on the four alternative tree topologies that varied in the ancestral state reconstruction. Because there were too many MPRs in arboreality and the morphological characters on these trees to examine concentrated changes in all MPRs in a reasonable amount of time, I tested for concentrated changes in both the case where arboreality was reconstructed to have evolved few times deep within the tree with more reversals (ACCTRANS reconstruction) and where arboreality evolved more times closer to the tips of the tree with fewer reversals (DELTRANS reconstruction). For the morphological characters, I examined each MPR or 10 randomly chosen MPRs if there were more than 10 for each phylogenetic tree and each MPR of arboreality. The number of origins within arboreal lineages and in the tree as a whole were counted, and the probabilities were determined using 10,000 simulations in MacClade. I considered only gains in the morphological characters because they are hypothesized to be adaptations for arboreality. When calculating the probabilities of concentrated changes, I included gains in the mor-

phological character that occurred on the same branch of the tree as a gain in arboreality. I also performed a more conservative analysis in which I considered only gains in morphological structures that unambiguously occurred after arboreality had evolved.

To search for correlated evolution between continuous traits and arboreality, I used the Brunch algorithm in Comparative Analysis by Independent Contrasts 2.6.8b (CAIC, Purvis and Rambaut 1995). This method tests the null hypothesis that changes in habitat have no effect on the particular morphological character examined. A *t*-test was performed on the contrasts at nodes of interest to assess whether a change in habitat was associated with a significant change in the values of the morphological measurements. The binary state of presence or absence of arboreality was used in this test because states in the multistate character of habitat could not be ranked in a transformation series with any certainty. The continuous morphological characters bulginess of eyes, prothorax shape, elytral shape, relative leg length, and number of teeth on tarsal claws were tested for correlations with arboreality on the strict consensus tree of the 32 MPTs in island 1, a strict consensus tree of 768 MPTs in island 2 of the 800 MPTs, and the minimum evolution distance tree.

Branch lengths for these trees were estimated from the 28S data and were constrained to be nonnegative. Zero length branches were set to a length of 10^{-8}. All tests were also done with all branches of equal length and with zero length branches set to 10^{-5} and 10^{-10}. For each tree, separate tests of correlations between arboreality and morphological characters were done for all data, data from males only, and data for females only.

RESULTS

Electronic Appendix, available from the *Evolution* Editorial Office at evolution@asu.edu, provides the taxa and data included in the analyses.

Evolution of Arboreality

When all MPRs of arboreality were examined, there were a minimum number of 21 independent origins on the 800 MPTs and a maximum number of 27 independent origins. The number of origins on the distance tree was similar (20–27, Table 1). Parsimony reconstructed as few as zero losses of arboreality in the MPTs and as many as six. On the distance tree, there were three to 10 losses of arboreality. The range in number of origins and losses of arboreality in the parsimony and distance trees is due to multiple MPRs of arboreality on each tree (32 for every parsimony tree and 112 for the distance tree), not to differences in tree topology.

The minimum and maximum number of origins and losses of arboreality reconstructed on the MPTs and the distance tree falls within the range reconstructed on the bootstrap trees (Fig. 4). There were no fewer than eight and as many as 36 origins of arboreality on the parsimony bootstrap trees. Parsimony reconstructed as few as zero and as many as 29 losses of arboreality on the parsimony bootstrap trees (Fig. 4). Results from the neighbor-joining bootstrap trees were similar (Fig. 4).

Table 2 shows the probability of different numbers of maximum origins and minimum losses of arboreality reconstructed using parsimony on trees from the Bayesian analysis. The data are most consistent with a maximum of 33 origins and four losses (0.1096) and 32 origins and four losses (0.0899). When the minimum number of origins and maximum number of losses were examined, a minimum of 27 origins and maximum of 10 losses (0.1201) had the highest posterior probability. The posterior probability of having overall more gains than losses of arboreality was 0.82; however, the posterior probability of having more than one loss was 0.97. The number of origins and losses with the highest probability in the Bayesian analysis was greater, for the most part, than in the 800 MPTs and the distance tree (Fig. 5), but consistent with the results of many origins and fewer losses of arboreality in all trees examined.

Maximum-likelihood estimation of the ratio of gains to losses in arboreality on the trees examined ranged from 0.98 to 2.262, depending on the state of taxa with polymorphic or uncertain states (Table 1). The rates of gains and losses did not differ significantly from each other ($P = 0.132$–0.958, $G = 0.003$–2.69, df = 1, Table 1), and the reversal rate back to ground-dwelling differed significantly from zero ($P < 0.0009$, $G = 7.192$–63.2060, df = 1, Table 1). The signifi-

TABLE 1. Character evolution in arboreality and discrete morphological characters reconstructed by parsimony and estimated by maximum likelihood (ML). Results of likelihood-ratio tests of rates of change and directionality of change in these characters.

| Character	MPRs	Parsimony gains	Parsimony losses	ML gain rate	ML loss rate	Gain rate = loss rate	Loss rate = 0
Most parsimonious tree(s)							
arboreality	32	21–27	0–6	5.6506–5.6926	2.5163–4.2852	$P \geq 0.4785$	$P \leq 0.0073$
elongated prothorax	2	13–14	0–1	2.4162	3.5691	$P = 0.7139$	$P = 0.0014$
expanded/bilobed tarsomere	19	14–17	2–5	4.4495	5.4156	$P = 0.6419$	$P < 0.0001$
adhesive setae	17	13–16	1–4	2.8080	2.3881	$P = 0.8254$	$P < 0.0001$
pectinate claws	216	6–24	0–19	2.4104	10.4977	$P = 0.0016$	$P = 0.0038$
Minimum evolution distance tree							
arboreality	112	20–27	3–10	3.5967–3.7426	1.7313–3.6869	$P \geq 0.1017$	$P \leq 0.0009$
elongated prothorax	2	16–19	0–3	2.4561	2.5128	$P = 0.1473$	$P = 0.005$
expanded/bilobed tarsomere	28	13–17	3–7	2.0887	2.3719	$P = 0.397$	$P \leq 0.0001$
adhesive setae	12	13–16	2–5	1.6760	1.7612	$P = 0.9292$	$P \leq 0.0001$
pectinate claws	8	11–17	5–11	1.5334	3.5484	$P = 0.0188$	$P = 0.0003$

Parsimony

Distance
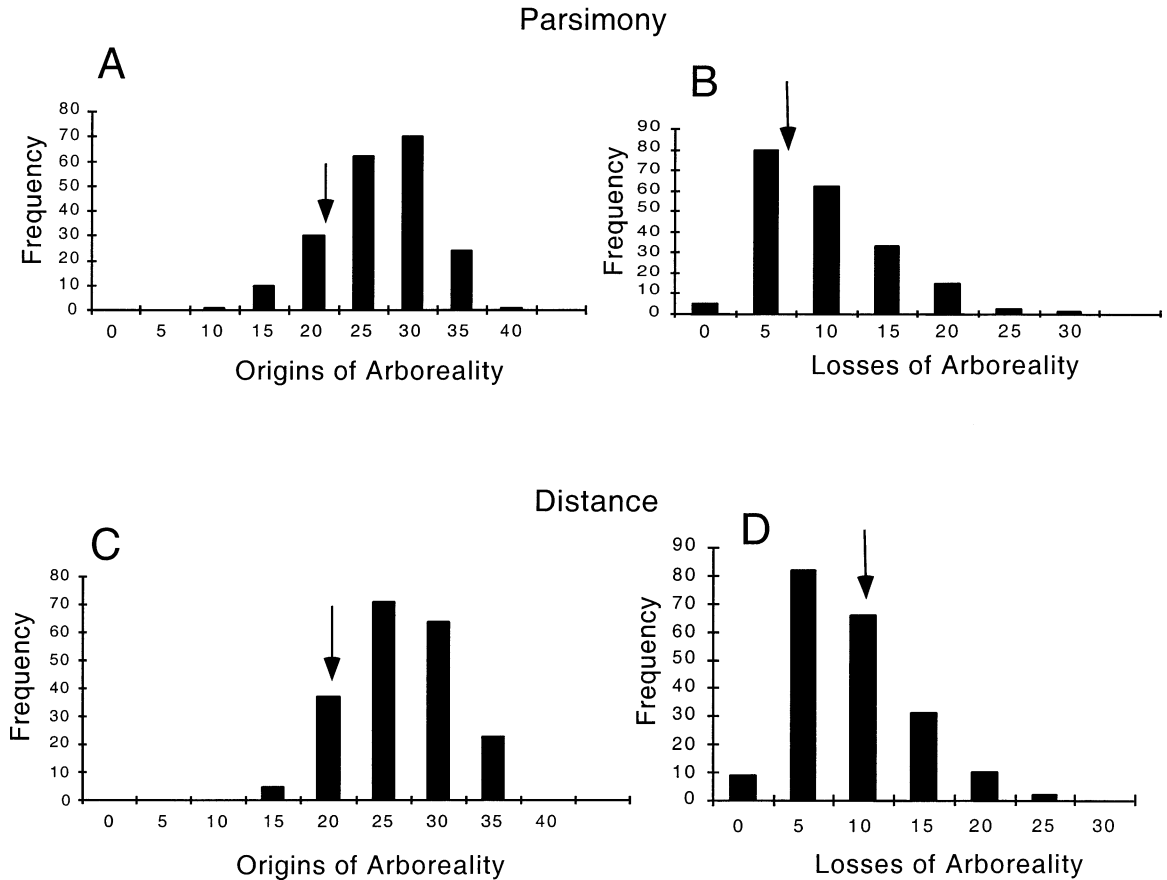
FIG. 4. The number of origins (A) and losses (B) of arboreality reconstructed with parsimony on trees from 100 parsimony bootstrap replicates from the 28S + *wingless* molecular data. The number of origins (C) and losses (D) of arboreality reconstructed with parsimony on 100 trees from neighbor-joining bootstrap replicates from the molecular data. The arrows indicate the minimum number of origins of arboreality reconstructed on the 800 most parsimonious trees (A) and the minimum evolution distance tree (C) from the molecular data; and the maximum number of losses reconstructed on the 800 most parsimonious trees (B) and distance tree (D).

cance of the results did not change with different branch length estimates.

Thirty-eight of the 68 arboreal taxa were classified as canopy specialists. Consideration of only these canopy specialists did not provide evidence for irreversibility of habitat specialization. The rate of origins of canopy specialists did not differ significantly from the loss rate in the parsimony tree ($P = 0.981$, $G = 0.000582$, df = 1) or the distance tree ($P = 0.991$, $G = 0.000122$, df = 1), but the loss rates did differ significantly from zero ($P < 0.015$, $G = 5.9176$–14.227, df = 1).

When ancestral states of the multistate habitat character were reconstructed on the 800 MPTs and the distance tree, no clear pattern of habitat transitions was seen (Table 3). Most changes were from a terrestrial habitat to other habitats in dead wood or on vegetation with relatively few reversals. Some notable exceptions were multiple transitions between terrestrial and corticolous habitats and back and between canopy and corticolous habitats. This pattern may be due, in part, to several polymorphic species that live on bark and tree trunks as well as in other habitats (e.g., species in the genera *Aenigma*, *Orthogonius*, *Diploharpus*, and *Pseudomorpha*) or to greater flexibility in habitat preference in corticolous harpalines.

Morphological Character Evolution

All discrete characters, with the exception of pectinate claws, had many gains and relatively few losses reconstructed by parsimony on the phylogenies (Table 1). For the pectinate claws character, in some MPRs losses were as many as or more than gains, and pectinate claws were reconstructed to have evolved deep within the harpaline clade, to have been lost in many clades, and then to have been subsequently regained by some taxa. Although the number of losses was typically less than the number of gains, most characters had maximum-likelihood loss rates that exceeded gain rates for both parsimony and distance phylogenies (Table 1). The gain and loss rates did not differ significantly from each other, however, for any character except pectinate claws (elongated prothorax: $P = 0.147$–0.714, $G = 0.0005$–0.1344, df = 1; expanded or bilobed tarsomeres: $P = 0.397$–0.642, $G = 0.2162$–0.7173, df = 1; and adhesive setae: $P = 0.825$–0.929, $G = 0.0079$–0.0487, df = 1;, Table 1). The pectinate claws loss rate was significantly greater than the gain rate for both the parsimony ($P < 0.002$, $G = 9.9977$, df = 1) and distance phylogenies ($P = 0.019$, $G = 5.5180$, df = 1). The loss rate for all morphological characters was significantly different from zero in both trees (Table 1), suggesting that reversals

TABLE 2. Posterior probabilities of phylogenetic trees from the Bayesian analysis having different numbers of origins and losses of arboreality reconstructed by parsimony. Bold value corresponds to the maximum number of origins and minimum number of losses of arboreality with the highest posterior probability. The 95% confidence interval includes a maximum of 30 to 37 gains and a minimum of two to seven losses.

Minimum number of losses of arboreality	Maximum number of origins of arboreality															
	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39
0													0.0005			
1											0.0044	0.0060	0.0072	0.0025	0.0040	0.0031
2									0.0245	0.0317	0.0372	0.0308	0.0129	0.0152		
3								0.0110	0.0899	0.0678	0.0736	0.0389	0.0061			
4							0.0020	0.0612	0.0538	**0.1096**	0.0290	0.0091				
5							0.0224	0.0399	0.0075	0.0220	0.0197	0.0001				
6					0.0008		0.0218	0.0290	0.0171	0.0428						
7				0.0030	0.0056		0.0246	0.0055								
8						0.0011										
9																
10																
11		0.0020	0.0003													
12		0.0002	0.0001													
13	0.0013															

FIG. 5. A histogram of the probability of the minimum number of origins of arboreality (A), maximum number of losses of arboreality (B), and minimum number of losses (C) from Bayesian analysis trees reconstructed with parsimony. A minimum of 27 gains has the highest posterior probability. The number of losses of arboreality with the highest posterior probability is a maximum of 10 losses (B) and minimum of four losses (C). m is the mean number of gains (A) or losses (B, C) reconstructed on the Bayesian trees; di is the number of gains (A) or losses (B, C) reconstructed on the distance tree; and pa is the number of gains (A) or losses (B, C) reconstructed on the parsimony trees.

in an elongated prothorax ($P < 0.005$, $G = 10.1925$–15.4813, df = 1), expanded or bilobed tarsomeres ($P < 0.0001$, $G = 25.1421$–39.5163, df = 1), adhesive setae ($P < 0.0001$, $G = 22.1529$–29.1633, df = 1), and pectinate claws ($P < 0.0038$, $G = 8.3962$–13.2056, df = 1) are likely. The different sets of branch length values used did not differ in the significance of the likelihood-ratio test results.

Correlated Morphological and Habitat Evolution

Among traits hypothesized to be adaptations for arboreality, only expanded or bilobed tarsomeres and adhesive setae were unambiguously associated with it. An elongated prothorax and teeth on tarsal claws showed a relationship only

TABLE 3. Most parsimonious reconstructions of changes in habitat in the most parsimonious trees and minimum evolution distance tree. Only one possible pathway for taxa with polymorphic or uncertain habitat states was considered.

	Change to				
Change from	Terrestrial	Subcanopy dweller	Canopy specialist	Dead wood dweller	Corticolous
Terrestrial	—	9–12	4–10	6–9	10–20
Subcanopy dweller	0–1	—	1–3	0	0–2
Canopy specialist	0–2	0–1	—	0–1	2–6
Dead wood dweller	0–1	0	0–1	—	0–3
Corticolous	0–7	0–2	3–5	0–4	—

in some phylogenies or some methods of analysis. Bulgy eyes, long elytra, and long legs were not correlated with arboreality.

Gains of expanded or bilobed tarsomeres ($P < 0.006$) and adhesive setae ($P < 0.007$) were concentrated in arboreal lineages in the MPTs and distance trees (Table 4). The correlated evolution of expanded or bilobed tarsomeres and adhesive setae with arboreality was also seen in the results of the maximum-likelihood estimates of correlated evolution. The correlated evolution model fit the data significantly better than an independent evolution model for both the parsimony ($P < 0.0001$, $G = 26.0$–51.0, df $= 4$) and distance trees ($P < 0.0001$, $G = 37.2$–41.2, df $= 4$; Table 4), even when alternative habitat states were used for taxa with polymorphic or uncertain habitat states. Significant results did not change with different branch length values.

Results of the test for concentrated changes in the binary character of an elongated prothorax with arboreality indicated that there was no significant association between prothorax shape and arboreality in the MPTs ($P = 0.2209$–0.3033). In the distance tree, an elongated prothorax was significantly concentrated in arboreal lineages in some of the MPRs ($P = 0.0004$–0.0842, Table 4). In the results of the maximum-likelihood correlated evolution tests, an elongated prothorax was significantly associated with arboreality in both the parsimony ($P = 0.0137$–0.0236, $G = 11.2768$–12.5530, df $= 4$) and distance trees ($P = 0.0089$–0.0393, $G = 10.0682$–13.5358, df $= 4$). When the character was coded as continuous, however, and analyzed with independent contrasts, there was no significant association between prothorax shape and arboreality ($P = 0.0854$–0.1614, $t = 1.4471$–1.7911, df $= 23$–25, Table 4).

Whether gains in pectinate claws were significantly concentrated on arboreal lineages depended upon the MPR of pectinate claws examined for the MPTs ($P = 0.0051$–0.5754). Pectinate claws were not concentrated in arboreal lineages in the distance tree ($P = 0.1051$–0.6533, Table 4). The number of teeth on the claws showed a significant increase with arboreality in only one of the islands of MPTs ($P = 0.0068$, $t = 2.9721$, df $= 23$, Table 4). The results of the maximum-likelihood correlation tests indicated a significant association between arboreality and pectinate claws for both parsimony ($P < 0.0002$, $G = 21.6$–23.9, df $= 4$) and distance trees ($P < 0.0008$, $G = 19.0$–25.3, df $= 4$; Table 4).

There was no significant correlation between arboreality and elytral L/W ($P = 0.5289$–0.9805, $t = 0.0247$–0.6393, df $= 23$–25), or relative leg length ($P = 0.1380$–0.6255, $t = 0.4908$–1.3159, df $= 22$–24, Table 4). Eyes were not sig-

nificantly more hemispheric (when evaluated with a Bonferroni correction) for the parsimony trees ($P = 0.0746$–0.0864, $t = 1.7915$–1.868, df $= 23$) or the distance tree ($P = 0.0397$, $t = 2.1705$, df $= 25$). Results of the all the independent contrasts were similar for all branch lengths examined and if all morphological data were used or males and females were examined separately (Table 5).

When seemingly simultaneous gains in morphological character states and arboreality (gains occurring on the same branch) were counted in the concentrated changes test and treated as evidence for a correlated relationship, I found a significant correlation between arboreality and the morphological characters of adhesive setae, expanded tarsomeres, and some MPRs of an elongate prothorax and pectinate claws. However, if only branches where a gain in arboreality precedes a gain in morphological characters are considered, the results for all comparisons are nonsignificant (Table 4). Maximum-likelihood tests were performed to examine whether arboreality was more likely to evolve on branches prior to a gain in morphological characters. In all correlations between arboreality and morphological characters, the parameter value indicating that a gain in arboreality occurred before a gain in the morphological trait was larger than the value for the parameter indicating that a change in the morphological trait occurred first. The difference in parameter values, however, was only significant in the cases of expanded tarsomeres and adhesive setae evolving after arboreality for the parsimony tree ($P \leq 0.0194$). When tests of whether gains in morphological traits were contingent upon arboreality were done, only gains in the characters of expanded tarsomeres and adhesive setae were significantly more likely when beetles are arboreal ($P \leq 0.0164$) in the parsimony tree. Results did not indicate whether gains in other morphological characters were significantly more likely with arboreality.

DISCUSSION

A variety of different methods was used to explore the evolution of arboreality in harpalines and the morphological characters commonly associated with it. None of these methods is without limitations. Parsimony methods are not guaranteed to infer the ancestral states of characters accurately. Parsimony can underestimate the number of changes in a character on the phylogeny, especially if there are high rates of change (Felsenstein 1985; Saitou 1989; Maddison 1994), and bias the ancestral states toward the more common state (King 1980). The difference seen in the loss rate in arboreality between the parsimony reconstructions and the maximum-

TABLE 4. Correlated evolution in discrete and continuous characters. Concentrated changes and likelihood-ratio tests were performed on discrete characters. Concentrated changes tests were done two ways: (1) to include morphological changes along branches that have a change in habitat; and (2) to include morphological changes only within arboreal lineages. Independent contrasts tests were done on continuous characters, and this accounts for missing entries in some cells of the table. An asterisk indicates a significant P-value when a sequential Bonferroni correction is applied.

Morphological character	Concentrated changes with arboreality	Concentrated changes within arboreal lineages	Maximum likelihood correlation with arboreality	Independent contrasts with arboreality
Most parsimonious tree(s)				
bulginess of eyes	—	—	—	$P = 0.075–0.086$
elongated prothorax	$P = 0.0812–0.2362$[2]	$P = 0.2109–0.3033$[2]	$P = 0.0137*–0.0236*$,[2]	$P = 0.101–0.161$[1]
elytral length/width	—	—	—	$P = 0.529–0.981$
relative leg length	—	—	—	$P = 0.202–0.629$
expanded/bilobed tarsomere	$P \leq 0.0059*$	$P = 0.1033–0.4535$	$P < 0.0001*$	—
adhesive setae	$P \leq 0.0068*$	$P = 0.3222–0.8346$	$P < 0.0001*$	—
pectinate claws	$P = 0.0051*–0.5754$	$P = 0.0842–0.6280$	$P < 0.0002*$	—
number of teeth on claw	—	—	—	$P = 0.007*–0.045$
Minimum evolution distance tree				
bulginess of eyes	—	—	—	$P = 0.040$
elongated prothorax	$P = 0.0004*–0.0842$[2]	$P = 0.1733–0.4612$[2]	$P = 0.0089*–0.0393*$,[2]	$P = 0.085$[1]
elytral length/width	—	—	—	$P = 0.632$
relative leg length	—	—	—	$P = 0.138$
expanded/bilobed tarsomere	$P \leq 0.0028*$	$P = 0.6322–0.9433$	$P < 0.0001*$	—
adhesive setae	$P \leq 0.001*$	$P = 0.2396–0.9528$	$P < 0.0001*$	—
pectinate claws	$P = 0.1051–0.6533$	$P = 0.2658–0.60$	$P \leq 0.008*$	—
number of teeth on claw	—	—	—	$P = 0.282$

[1] Coded as a continuous character, prothorax length/width.
[2] Coded as binary character.

likelihood estimations may be due to parsimony's underestimation of changes back to ground-dwelling.

An advantage of independent contrasts and maximum-likelihood Markov models in analyzing character evolution is the incorporation of branch length information into estimates of rates of change and tests of correlated character evolution. However, these methods can be sensitive to inaccuracies in branch lengths (Garland et al. 1992; Pagel 1994; Purvis and Rambaut 1995). Additionally, the branch lengths estimated from the 28S data might not accurately describe the rate of change in the morphological and habitat characters in the phylogeny. The overall results of independent contrast tests for character correlation and the maximum-likelihood estimates of rates of change and correlated evolution in characters did not change when branches were assessed in different ways.

In general, the results of the character evolution analyses were not sensitive to method of analysis, and different phylogenetic topologies did not significantly affect the results of the character evolution analyses. The maximum-likelihood analyses gave higher estimates of character loss rates overall, whereas the minimum losses reconstructed by parsimony were low. However, even if the maximum-likelihood estimated rate of change for a character is greater than zero, parsimony would not necessarily reconstruct a loss, even if a loss actually happened on the tree. In spite of somewhat ambiguous results from the analyses, a few hypotheses about the evolution of arboreality in carabids can be made.

Evaluation of the Taxon Pulse Model and the Evolution of Arboreality

Two predictions of the taxon pulse model for the evolution of arboreality in carabids were tested: (1) arboreal lineages do not have terrestrial descendants; and (2) change in one or more morphological adaptations for arboreality is irreversible. Neither of these predictions is supported by the data, and thus the taxon pulse model is rejected. Reversals from arboreality to ground-dwelling occurred in all the phylogenies examined. Although the number of reversals tends to be smaller than origins, the reversal rate is greater than zero and may be as high as the gain rate. In addition, reversals in morphological adaptations to arboreality also occurred.

There have been many origins of arboreality in harpalines, clearly more than a single origin, and perhaps more than 20. The move onto vegetation and into the specialized habitat of the rainforest canopy may have been influenced by a number of factors. The increasing complexity of flowering plants and development of tropical canopies in the Cretaceous offered a new niche for insects, including speciating carabid groups (Erwin and Pogue 1988). Seasonal flooding in lowland forests, caused by changes in climate, may have favored carabids that moved up onto vegetation and then into the canopy (Erwin and Adis 1982). Severe predation pressures from the birds, frogs, and lizards and intense competition for resources with other carabids, as well as with the radiation of ants and rove beetles, may have influenced the evolution of arboreality in carabids (Erwin 1979).

Reversals from arboreality to a terrestrial way of life have been common in harpalines. Because the taxon sampling of

TABLE 5. Correlated evolution of continuous morphological characters with arboreality on trees with branch lengths estimated from molecular data.

| | Morphological character | Independent contrasts with arboreality | |
		Males only	Females only
Most parsimonious trees	bulginess of eyes	$P = 0.071–0.094$	$P = 0.144–0.147$
	prothorax length/width	$P = 0.098–0.134$	$P = 0.206–0.242$
	elytral length/width	$P = 0.110–0.140$	$P = 0.437–0.50$
	relative leg length	$P = 0.585–0.741$	$P = 0.580–0.678$
Minimum evolution distance tree	bulginess of eyes	$P = 0.098$	$P = 0.038$
	prothorax length/width	$P = 0.134$	$P = 0.144$
	elytral length/width	$P = 0.152$	$P = 0.503$
	relative leg length	$P = 0.59$	$P = 0.304$

all terrestrial and arboreal lineages of harpalines was not complete in this study, more reversals from arboreality to ground-dwelling still may be found and may be even more common than previously predicted. This result is in conflict with the taxon pulse model (Erwin 1979) of carabid evolution into specialized habitats. The taxon pulse proposes that carabid evolution in specialized habitats, like rainforest canopies, is unidirectional and that reversals would be rarely or never seen; lineages in specialized habitats are destined for extinction and replacement by subsequent groups of carabids. Evidence against the taxon pulse was also found in patterns of reversals in the morphological characters that have been thought of as specialized adaptations for arboreality. These features were thought to be so specialized to their habitats that reversal to more generalized forms would be unlikely (T. Erwin, pers. comm.). The maximum-likelihood estimations of rate and direction of evolution of arboreality and canopy specialists from this study suggest that the taxon pulse does not accurately describe the evolution of arboreal harpalines.

Most origins of arboreality are close to tips of the tree and not found in very deep nodes of Harpalinae. Sparse and non-random (dispersed) taxon sampling may be one explanation for this pattern. If more arboreal lineages of harpalines were included, then the origins of arboreality may be reconstructed deeper in the tree. If more terrestrial harpalines were included, more reversals to ground-dwelling might be observed. The taxon sampling strategy employed in this study sought to avoid such pitfalls. The pattern, however, may be the result more of biological process than sampling artifact. Arboreality in most lineages of harpalines may have evolved relatively recently, and these lineages are relatively young and have not had the opportunity to diversify. Alternatively, arboreal lineages may frequently go extinct after they become arboreal, such that they are not long-standing or persistent deep in the harpaline phylogeny. This last hypothesis, if true, would partially support the taxon pulse model of carabid evolution.

The taxon pulse, in its most strict form (where no reversals to terrestrial habitats are possible), is not an accurate model for the evolution of arboreality. Reversals from arboreal habitats and even canopy specialist habitats were observed in harpalines. However, in general there were fewer losses of arboreality than origins. Although reversals are possible, they may not be frequent. A revised model of the taxon pulse into arboreal habitats must include at least occasional reversals.

Arboreality and Morphological Evolution

Morphological features have coevolved repeatedly with arboreality in independent lineages of harpalines. Expanded or bilobed fourth tarsomeres and adhesive setae on the tarsomeres are adaptations to arboreality, implicating natural selection as the cause of morphological evolution. The correlation between these morphological characters and the arboreal habit was seen across different lineages of harpalines. Specialized tarsal adhesive setae are also found in many arboreal Cerambycidae and Chrysomelidae (Stork 1980; Eisner and Aneshansley 2000) and are used mainly to aid climbing on smooth regions of plant surfaces (Stork 1983) like many tree-dwelling lizards (Cartmill 1985; Autumn et al. 2002). Insects can improve their adhesion to plants, probably through van der Waals forces (Stork 1980), with an increased number of adhesive setae borne on broad tarsomeres that can accommodate a higher number of setae (Stork 1980, 1987).

Adhesive setae and expanded or bilobed tarsomeres and arboreality seem to evolve simultaneously, implying strong selection for morphological change in certain habitats and a close and rapid connection between changes in habitat and changes in morphology. Alternatively, arboreal species without the morphological characters (or vice versa) are now extinct or have not been sampled. Caution must be taken when interpreting the results from the concentrated changes tests because the order of events (cause and effect) in changes in arboreality and morphological characters cannot be determined on branches where both habitat and morphological characters change (Donoghue 1989). In this case, because these characters are so tightly correlated, the results depend critically on how simultaneous appearances of characters on a branch are interpreted. It appears from the maximum-likelihood tests that arboreality evolved before expanded tarsomeres and adhesive setae, and that these traits were more likely to evolve in the presence of arboreality. However, there is no strong evidence from the temporal order and contingent change tests with maximum-likelihood that arboreality evolved prior to the other morphological characters (or vice versa), perhaps because these characters have changed nearly simultaneously in evolutionary history.

There is no evidence that elongated elytra, relatively long legs, and bulgy eyes are correlated with arboreality. Arboreal harpalines have a notable diversity of body shapes, from the small and wide *Lebia* and *Hyboptera* to the long and thin *Ophionea* and *Agra*. Selection on the above characters may

depend on arboreal microhabitat, such as leaves versus twigs and tree bark versus grass stems, but I did not test for such correlations here. For instance, Habu (1967) suggested that different leg forms adapt carabids for life on grass stems, tree trunks, or leaves.

The association of an elongated prothorax and pectinate claws with arboreality is unclear. Prothorax shape varies from very wide (e.g., *Hyboptera*) to very narrow (e.g., *Ophionea*) among arboreal carabids. An elongated prothorax is also commonly found in terrestrial harpalines, especially in cave-dwelling beetles like some zuphiines and platynines (Barr and Lawrence 1960; Moore 1995) and in carabids that live in crevices of rocks, wood, or soil (Erwin 1979). Such a variety of prothoracic shapes among arboreal and terrestrial carabids could mask the ability to detect a correlation of an elongated prothorax with arboreality if it occurs. Functional performance tests may be needed to better understand the fitness consequences of an elongated prothorax for arboreal carabids. Whether pectinate claws evolve in conjunction with arboreality is ambiguous. Reversals seem very common in this character. Casale (1988) showed in his revision of the carabid subtribe Sphodrina that smooth claws are a possible, simple reversal from the pectinate state. Teeth on the tarsal claws are one of the most common features of arboreal carabids (Stork 1987); however, pectinate claws are seen widely in harpalines, even among terrestrial species (e.g., *Anaulacus* and *Microlestes*), and thus may be associated with many habitat types including sand and loose soil. Even different species of the same genus show variation in the presence or absence of teeth (e.g., *Stenognathus*, Reichardt 1977) or number of teeth (e.g., *Orthogonius*, Electronic Appendix; *Dromius*, Stork 1987). However, an increase in number of teeth may be correlated with arboreality (Table 4), implying that more teeth on the claws may enable better vertical climbing on plant surfaces.

While some transitions to new lifestyles or niches are unidirectional, the evolutionary adaptation to a particular mode of life or habitat may not be a dead end. Phylogenies are important for reconstructing the patterns and processes of ecological and morphological change and for testing hypotheses of the reversibility of habitat shifts. Several studies have elucidated similar patterns of reversals from derived lifestyles or habitats. Habitat reversals occur in springtails (D'Haese 2000). The troglobitic habit in crickets is not an obligatory evolutionary dead end and can give rise to surface living (Desutter-Grandcolas 1997). Reversals to marine habitats have occurred among insects and other ancestrally terrestrial arthropods; however, they are rare (Vermeij 2000). Even for parasitic modes of life that are often considered irreversible, evolutionary shifts from parasitism toward free-living habits can be found in the case of Diplomonadina (Siddall et al. 1993) and Nematoda (Blaxter et al. 1998). Marvaldi et al. (2002) suggest that weevils ancestrally associated with gymnosperms shifted to angiosperm hosts multiple times and, in some groups, reversed back to gymnosperm feeding. They also found that, whereas some feeding habits are irreversible (feeding on leaves or seeds), reversals are seen in other kinds of feeding habits (stem and trunk boring). Schluter (2000) and Nosil (2002) both observed trends toward specialization from generalist ancestors in

many different kinds of organisms, but suggested that the trend is not universal or widespread. Reversal may be a common evolutionary process, and evolution of new ecological interactions or evolution into new habitats may not inhibit further evolution or reversals.

The evolutionary process of unidirectional character change may not be as widespread or absolute as previously thought. A milder version of Cope's law of the unspecialized may be more appropriate for many cases of character change. Reversals to ancestral character states can provide insights into evolutionary processes and should be critically examined. Understanding the direction and restrictions on changes can shed light on the causes of evolutionary change.

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