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E-Article

The Tempo and Mode of Three-Dimensional Morphological Evolution in Male Reproductive Structures

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abstract: Various evolutionary forces may shape the evolution of traits that influence the mating decisions of males and females. Phenotypic traits that males and females use to judge the species identify of potential mates should evolve in a punctuated fashion, changing significantly at the time of speciation but changing little between speciation events. In contrast, traits experiencing sexual selection or sexually antagonistic interactions are generally expected to change continuously over time because of the directional selection pressures imposed on one sex by the actions of the other. To test these hypotheses, we used spherical harmonic representations of the shapes of male mating structures in reconstructions of the evolutionary tempo of these structures across the history of the *Enallagma* damselfly clade. Our analyses show that the evolution of these structures is completely consistent with a punctuated model of evolutionary change and a constant evolutionary rate throughout the clade's history. In addition, no interpopulation variation in shape was detected across the range of one species. These results indicate that male mating structures in this genus are used primarily for identifying the species of potential mates and experience little or no selection from intraspecific sexual selection or sexual antagonism. The implications of these results for speciation are discussed.

Keywords: mating structures, morphological evolution, punctuated change, reproductive isolation, spherical harmonics.

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To understand fundamental questions about species diversity, we must understand why individuals are the same or different species. As codified by the biological species concept (Mayr 1942), speciation in sexual organisms is at its core the set of processes that generates reproductive isolation among groups of individuals (Dobzhansky 1937, 1940; Mayr 1942). Reproductive isolation is generated by the differentiation of myriad possible traits that may disrupt mating, zygote formation, or offspring performance between sets of individuals (Howard and Berlocher 1998; Coyne and Orr 2004). Some species are reproductively isolated from one another because they produce inviable or infertile offspring (Noor and Feder 2006). Others are reproductively isolated because of differences in proteins that prevent gamete recognition or fusion (e.g., Swanson and Vacquier 2002; Springer and Crespi 2007). Still others will not mate with one another because of female discrimination based on calls (e.g., Henry et al. 1999; Shaw 2000; Gerhardt 2005), pheromones (e.g., Blows 2002), behavioral repertoires (e.g., Boake 2002), or morphological compatibility (Eberhard 1985; Shapiro and Porter 1989). The evolution of traits involved in these reproductive isolating mechanisms define the tempo and mode of speciation that create biological diversity.

In many insects and other arthropods, morphological features of genitalia and other reproductive structures are thought to define reproductive compatibility (Eberhard 1985). In many arthropod taxa, species are defined by morphological features of these reproductive structures (Eberhard 1985), and these species definitions based on morphological definitions typically stand the test of molecular data in defining species boundaries (e.g., Turgeon et al. 2005). Such species specificity is thought to be indicative of the role these structures play in enforcing reproductive isolation and allowing males and females to identify individuals as conspecifics or heterospecifics at the time of mating (e.g., Paterson 1978, 1993; Templeton 1979). For example, the "lock-and-key" hypothesis (Dufour 1844) postulates that the match or mismatch between male and female reproductive structures enforces preinsemination reproductive isolation (Shapiro and Porter

1989; Arnqvist 1997). If evolutionary changes in these structures are the defining acts of creating new species and they enforce reproductive isolation after differentiation, they should show a characteristic pattern of evolution over the history of a clade; namely, these characters should change rapidly at the time of speciation but change little between speciation events because of stabilizing selection for species mate recognition (Eberhard 1985; Paterson 1993; Arnqvist 1997; Hosken and Stockley 2003). Stabilizing selection for mate recognition should also favor large morphological differences between species relative to intraspecific differences (Gavrilets 2000; McPeek and Gavrilets 2006).

Although such evolutionary dynamics are usually presumed for these structures, these presumptions are based more on inference than on explicit tests (Huber 2003). For example, the fact that taxonomists can use these characters to delineate species is often taken as evidence for these dynamics (Eberhard 1985; Huber 2003). However, other hypotheses have also been advanced to explain the evolution of these traits, and these alternatives do not necessarily imply such rapid evolutionary change associated with speciation events. The two most common alternatives are sexual selection and sexually antagonistic coevolution (Eberhard 1985, 2004, 2005; Andersson 1994; Arnqvist 1997). Both postulate that interspecific differences are simply an extension of the consequences of intraspecific interactions between or within the sexes. Sexual selection via female choice or sperm competition among males should favor particular features of male structures that will increase male mating success (e.g., Eberhard 1985; Andersson 1994; Bertin and Fairbairn 2005). Also, male structures may be favored that exploit "sensory traps" of females to increase their own mating success (e.g., Córdoba-Aguilar 2002). In contrast, sexually antagonistic coevolution postulates that male and female traits evolve in an evolutionary arms race in which male characters evolve to overcome female defenses to reduce copulations while female traits evolve to elaborate these defenses (Arnqvist et al. 2000; Arnqvist and Rowe 2005). Unlike species recognition, both of these hypotheses postulate that the dynamics of character change are a much more continuous process of directional selection to elaborate these traits, and the operation of these mechanisms is not restricted to the period in which new species are formed (Andersson 1994; Arnqvist and Rowe 2002*a*, 2000*b*).

These contrasting hypotheses for the evolution of genitalia and other sexual traits predict alternative tempos of character evolution. Characters that are primarily species identifiers to potential mates should show punctuated character change at the time of speciation but change little within lineages between speciation events because of strong stabilizing selection imposed by species mate recognition. In contrast, characters that experience strong intraspecific sexual selection or sexual antagonism should display a more continuous change over the history of a clade because directional selection generated by these interactions should operate nearly continuously. In this article, we test these conjectures for the male cerci—the morphological structures that males use to grasp females at the time of mating—of *Enallagma* damselflies (Odonata: Coenagrionidae). Cerci are the diagnostic morphological structures used to identify males to species (Westfall and May 2006), and previous experimental results have shown that females use cerci morphology to evaluate whether males are suitable mates (Paulson 1974; Robertson and Paterson 1982). To do this, we apply a new analytical methodology, spherical harmonics, to quantify three-dimensional shape variation in the cerci of 41 *Enallagma* species. We then use modifications of evolutionary contrasts analyses to reconstruct the tempo of evolutionary change in shape across the history of the genus.

Study System

Enallagma is one of the most speciose genera of odonates (Westfall and May 2006). The clade has 38 Nearctic species distributed over North and South America and the Caribbean Islands and four Palearctic species that range from north Africa and the Iberian Peninsula to Japan and the Kamchatka Peninsula of Asia (Westfall and May 2006). Previous phylogenetic analyses based on mtDNA sequences date the last common ancestor for the genus to about 15 million years ago (Brown et al. 2000; McPeek and Brown 2000; Turgeon et al. 2005).

Six clades with relatively distinct evolutionary histories can be distinguished within the genus (fig. 1). The "*hageni*" and "*carunculatum*" clades each radiated within the last 250,000 years. These radiations produced many species in each that now have broad and overlapping ranges extending over much of North America and a few species in each that are endemic to the Atlantic coastal plain (we refer to these as the *hageni* and *carunculatum* clades because these species appear to be the progenitors of the respective radiations; Turgeon et al. 2005). The four Palearctic species are the result of a third radiation that is sister to the *hageni* clade (Turgeon et al. 2005). These Palearctic species have largely allopatric ranges.

Two other clades have much longer evolutionary histories. The orange clade (adults of these species are shades of red, orange, or yellow) contains three lineages that each speciated within the same time period as the *hageni* and *carunculatum* clades. Two of these lineages contain pairs of species, one with a very large range covering much of eastern North America (*Enallagma signatum* and *Enallagma vesperum*) and the other an Atlantic coastal plain

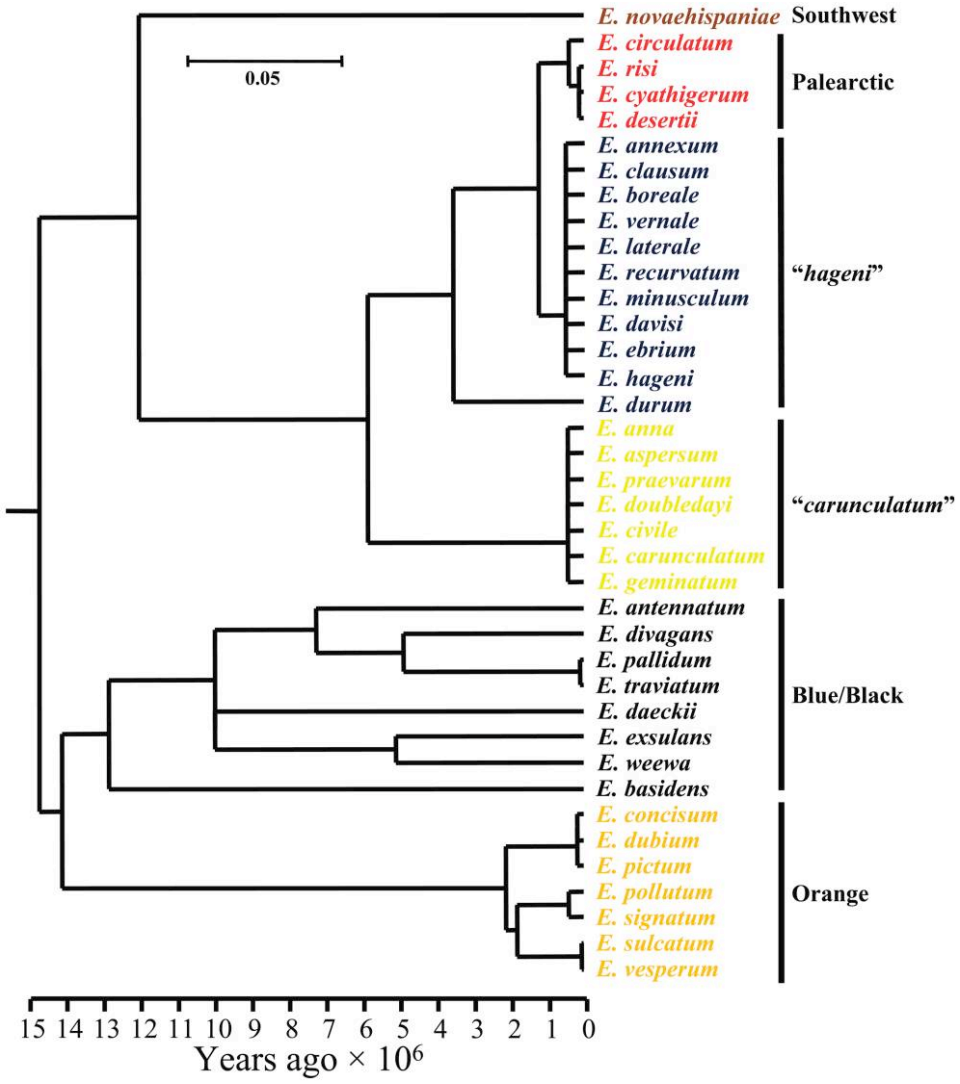


Figure 1: Phylogeny of the *Enallagma* damselflies taken from Turgeon et al. (2005). Branch lengths are proportional to time and have previously been calibrated to molecular clock estimates for insect mitochondrial DNA evolution (see Turgeon et al. 2005 for methods). Each of the major clades in the genus is identified to the right of the tree, and the names of extant species members are color coded.

endemic (*Enallagma pollutum* and *Enallagma sulcatum*). The third contains three Atlantic coastal plain endemics. The last common ancestor of the orange clade dates to about 2 million years ago (McPeek and Brown 2000; Turgeon et al. 2005). The blue/black clade (adults of most of these species are colored blue and black) is by far the oldest, with its last common ancestor dating to about 13 million years ago (McPeek and Brown 2000; Turgeon et al. 2005). Most of these species have ranges covering much of eastern North America, with two (*Enallagma pallidum* and *Enallagma weewa*) being Atlantic coastal plain endemics.

A sixth, Southwest clade contains *Enallagma novaehispaniae*, *Enallagma eiseni*, and *Enallagma semicirculare* in the southwestern United States and Mexico; *Enallagma coecum* in the Caribbean; and *Enallagma cardenium* in Florida. We only have enough mtDNA data to place one on the full *Enallagma* phylogeny (fig. 1), but smaller mtDNA fragments place the other species with *E. novaehispaniae* (M. A. McPeek, unpublished data). One final species (*Enallagma truncatum*) is endemic to Cuba, and its phylogenetic affinity to these six clades is unknown.

Species that are scattered across the phylogeny interact at lakes across North America. In eastern North America,

up to 12 species can be found at any one lake, and representatives of the *hageni*, *carunculatum*, blue/black, and orange clades are common at most (McPeek 1990, 1998). Moreover, up to seven species can be breeding at a lake on any one day (M. A. McPeek, unpublished data).

Enallagma have a fascinating breeding system. Females spend almost all their time foraging away from ponds to garner resources for egg production, and they only return to ponds to oviposit (Fincke 1982). This minimizes their exposure to male harassment and suggests an important role for sexual conflict in the evolution of mate choice. In contrast, males spend all their time at ponds searching for females; males are not territorial. Thus, operational sex ratios at ponds are highly skewed toward males (Fincke 1982, 1986*a*). When a female returns to oviposit, she is swarmed by males of more than one species who struggle to gain the proper hold on her to initiate mating. Females are sometimes injured and occasionally even killed during these male struggles. Males show little discrimination among females of different species and thus will try to gain control of females of most species (Paulson 1974; Fincke et al. 2007). A single male wins control of a female when he grasps her thorax with his terminal abdominal appendages: two superior cerci and two inferior paraprocts (fig. 2*a*). The male's paraprocts grasp the posterior dorsal surface of her prothorax, and the cerci grasp the anterior dorsal surface of her mesothorax, including her mesostigmal plates. If she accepts him, the male bends his abdomen and deposits sperm, which exits his body on the underside of the ninth abdominal segment to his penis on the underside of the second abdominal segment while he still holds her. The female then bends her abdomen up to be inseminated. During copulation, the male deposits his sperm after using his penis to remove sperm of previous matings (Waage 1979; Fincke 1984; Córdoba-Aguilar et al. 2003). They then return to the water, where the male releases her, and she climbs down a plant stem to oviposit underwater (Fincke 1986*b*). A male cannot force a female to bend her abdomen, and so females cannot be forcibly inseminated—females exercise ultimate choice of their mating partners (Fincke 1997).

The morphological shapes of the males' cerci and the females' mesostigmal plates are the diagnostic structures used by humans to identify individuals with species (Westfall and May 2006). Damselflies also seem to use these structures to identify one another with species during reproduction (Paulson 1974; Robertson and Paterson 1982). Typically, a female will not mate with a heterospecific male, and he will release her in less than 2 min (M. A. McPeek, personal observation). Females also refuse conspecific males with altered cerci just as they do heterospecific males, but they show no preference between conspecific males with surgically altered and unaltered paraprocts (Robertson and Paterson 1982). The specific mate recognition system driven by these structures appears to be very efficient. A genetic survey of the 17 species in the *hageni* and *carunculatum* clades using amplified fragment length polymorphism (AFLP) loci found evidence for hybridization between only two pairs of species: between *Enallagma hageni* and *Enallagma ebrium* and between *Enallagma boreale* and *Enallagma annexum* (Turgeon et al. 2005). All other species were mutually reproductively isolated from one another. Thus, male cerci and female mesostigmal plates appear to be key components of premating reproductive isolation among *Enallagma* species.

Material and Methods

For this study, we obtained specimens of all but two species of the *Enallagma* damselfly clade (Odonata: Coenagrionidae; table A1). The only species we lacked were *Enallagma deserti* from north Africa and *Enallagma truncatum* from Cuba. Most specimens included in this study were originally preserved by drying. A few specimens were originally preserved in ethanol; these ethanol-preserved specimens were first dried at 60°C for 24 h and then included. We found no difference in cerci morphology due to preservation methods.

Quantifying Cerci Size and Shape

Each specimen was scanned using computer tomography (CT) technology in a SkyScan 1172 high-resolution micro-CT scanner (SkyScan, Kontich, Belgium). The abdomen of a male was mounted on a brass stub using plastic tubing and modeling clay and was placed in the scanner. Computer tomography scans were made at a pixel resolution of 2.5 μm (i.e., voxel resolution of 15.6 μm^3) through 180° with a rotation step of 0.7°/frame and averaging three frames. Computer tomography scans were converted to stacks of digital image slices using NRecon, version 1.4.4 (SkyScan).

The left and right cerci were segmented from the resulting digital image stack, and initial processing was performed using Amira, version 4.1.2 (Mercury Computer Systems, Chelmsford, MA). All voxels associated with each cercus were first identified using the editing and labeling tools. Because we were only interested in reconstructing the exoskeletal surfaces of the cerci, we closed off the anterior opening by adding a flat sheet of voxels and then filling the volume. We did this so that our models only reconstructed the outer surface of each cercus (see fig. 2*b*, anterior view). A high resolution triangular mesh surface model of each structure was then constructed. For computational purposes, each triangular mesh surface model was reduced to have 10,000 triangles with 5,002 vertices.

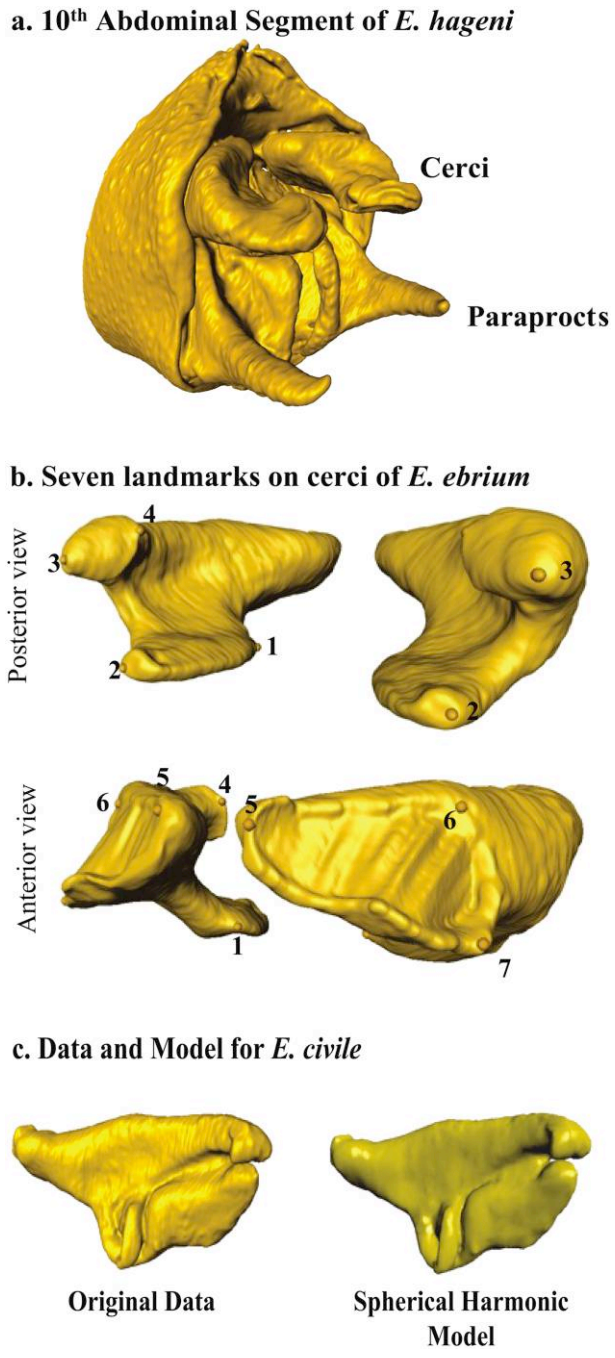


Figure 2: Computer-generated models of male damselfly structures derived from computer tomography scans. *a*, The four appendages at the end of an *Enallagma hageni* male's abdomen that he uses to grasp females before mating. The two superior appendages (cerci) contact the mesostigmal plates of a female's mesothorax. These are the structures used by females to identify males by species before mating. The two inferior appendages (paraprocts) contact the females prothorax. *b*, The seven landmarks used to register cerci positions for analysis are identified with small dots on the surface of the cerci of *Enallagma ebrium*. The left and right cerci are shown. The upper view is looking at the cerci from a view that is behind the animal, and the lower view is looking at the same pair of cerci rotated so as to view the surfaces where the cerci are attached to the rest of the body. *c*, The left image shows the raw data surface for the right cercus of *Enallagma civile*, and the right image shows the spherical harmonic model generated from these data.

The positions of seven landmarks were also identified on each cercus (fig. 2*b*). Three landmarks (*5–7*) identified proximal points on the cercal surface where it attaches to the body. The other four (*1–4*) identify points on the distal cercal surface (fig. 2*b*). These landmarks were used to register cerci relative to one another.

We also used Amira to calculate three measures of "size" for each cercus. A linear measure of size was calculated as the longest length from the most proximal and axial landmark (*5* in fig. 2*b*) to any of the four distal landmarks (*1–4*). We also calculated the surface area and volume of each cercus as two other measures of size.

We used spherical harmonics analyses of the triangular mesh surfaces to quantify cerci shape (Shen and Makedon 2006). Spherical harmonics, an extension of the classic Fourier transform, represent a three-dimensional (3-D) shape in terms of a sum of 3-D sines and cosines on a sphere (Brechbühler et al. 1995; Ritchie and Kemp 1999; Shen et al. 2007). We calculated the spherical harmonic representation of each surface using the algorithms described by Shen and Makedon (2006). Here, we provide only a general overview of the methods; for a more detailed description, consult Shen and Makedon (2006) and references therein.

Spherical harmonics form a complete set of orthonormal functions (sines and cosines) and thus form a vector space analogous to canonical basis vectors. On the unit sphere, any square integrable function can be expanded as a linear combination of these basis functions. A 3-D surface is parameterized in polar coordinates in terms of the functions $x(\theta, \varphi)$, $y(\theta, \varphi)$, and $z(\theta, \varphi)$ for $\theta, \varphi \in [0, \pi]$. Each of these functions is then expanded in terms of the spherical harmonics Y as follows:

$$x(\theta, \varphi) = \sum_{l=0}^{\infty} \sum_{m=-l}^{l} a_l^m Y_l^m(\theta, \varphi), \quad (1)$$

$$y(\theta, \varphi) = \sum_{l=0}^{\infty} \sum_{m=-l}^{l} b_l^m Y_l^m(\theta, \varphi), \quad (2)$$

$$z(\theta, \varphi) = \sum_{l=0}^{\infty} \sum_{m=-l}^{l} c_l^m Y_l^m(\theta, \varphi), \quad (3)$$

where the spherical harmonic basis functions are defined as

$$Y_l^m(\theta, \varphi) = \mathrm{N} e^{im\varphi} P_l^m(\cos\theta), \quad (4)$$

where N is a normalization constant and P_l^m is a Legendre polynomial. The coefficients a_l^m, b_l^m, and c_l^m embody the contributions of different spatial frequencies to the underlying surface and can be used to reconstruct the original surface, compute the "distance" between two surfaces, and morph from one shape to another. The coefficients are estimated by solving a series of linear equations using standard least squares estimation. These coefficients typically are complex numbers. Including higher-frequency components (i.e., greater l) models greater detail of the structure. These coefficients are estimated by solving a series of linear equations using least squares methods. The *Enallagma* cerci were modeled to degree $l = 15$, which produces 256 ($= [l + 1]2$) coefficients to represent each of the three dimensions for a total of 768 complex-valued coefficients. These algorithms were coded and run using Matlab, version 7.4.0.287 (MathWorks, Natick, MA); this code is available from the authors on request.

Before estimating the spherical harmonic coefficients, we standardized all cerci to a common length measure in order to remove overall size from these representations. We chose to standardize all cerci to a common length measure, because the contact between male cerci and female mesostigmal plates depends on distances between various points on the cerci, whereas total surface area and volume have much less influence on the proper contacting of these structures (M. A. McPeek, unpublished data). To do this, we rescaled the 3-D positions of the vertices in each triangular mesh so that the length measure used above was 1.0. Using other measures to standardize for size (e.g., using the distance between two landmarks consistently, square root of surface area) did not, however, alter the conclusions drawn from the analyses.

Intraspecific Variation

To assess the degree of intraspecific variation in cerci morphology, we scanned 41 individuals from five populations of *E. hageni*. These included 17 individuals from Lovewell Pond, Fryeburg, Maine (collected in June 1995: N43°59.99′, W70°56.14′); five individuals from Three Lakes II, Richland, Michigan (collected in June 2000: N42°20.95′, W85°25.78′); nine from Deep Lake, Hastings, Michigan (collected in June 2001: N42°37.35′, W85°27.43′); four from Blackhawk Lake Wildlife Management Area, Lake View, Iowa (collected in June 2001: N42°17.72′, W95°2.96′); and six from the Upper St. Croix Lake, Solon Springs, Wisconsin (collected in July 2001: N46°22.95′, W91°46.87′). Individuals from the Maine, Iowa, Wisconsin, and Deep Lake Michigan populations were also photographed. Head widths, forewing lengths, and abdomen lengths were measured from these photographs using Image-Pro Plus, version 6.2 (Media Cybernetics, Bethesda, MD).

We evaluated whether these populations of *E. hageni*

differed in cerci size or shape using MANOVAs (Morrison 2004). One analysis included the three size measures (i.e., length, surface area, volume) as response variables, with population as a random independent variable. We also correlated these measures of cerci size with the other body measurements. In order to reduce the dimensionality of the spherical harmonic representation, we applied principal components (PCs) analyses of the complex spherical harmonic coefficients (PCs extracted from the covariance matrix). We then performed a MANOVA on the first 10 PCs to test for shape differences among the populations. Statistical analyses were performed using SAS for Windows, version 8.02 (SAS Institute, Cary, NC).

Tempo of Cerci Evolution

Evolutionary contrasts analyses were then used to quantify evolutionary rates of change in cerci shape by reconstructing the spherical harmonic coefficients along the phylogeny of the *Enallagma*. Standardized evolutionary contrasts were calculated according to the algorithm first expounded by Felsenstein (1973, 1985; see also Garland et al. 1999; Rohlf 2001; Blomberg et al. 2003). A standardized evolutionary contrast is the difference in phenotype between two species that share an immediate common ancestor divided by the square root of the sum of branch lengths leading from this common ancestor to these two species. Branch lengths are meant to quantify a measure of "time" that is appropriate for the evolution of the character. Thus, a standardized evolutionary contrast measures the rate (i.e., distance/time) of character evolution in that portion of the phylogeny (Garland 1992; McPeek 1995*a*, 1995*b*).

In this article, we introduce a new type of standardized evolutionary contrast—the multivariate evolutionary contrast. In ordinary analyses, separate sets of contrasts are calculated for each variable in the data set, and the covariance structure is maintained among the variables in the set. Consider a set of n species for which a phylogeny with branch lengths has been developed. For each of these n species, two characters, X_i and Y_i (i indexes species $i = 1, \ldots, n$), have been measured. Standardized evolutionary contrasts are calculated by taking the difference in phenotype between pairs of species that share an immediate common ancestor on the phylogeny and dividing this difference by an estimate of evolutionary time (e.g., the square root of the sum of the branch lengths from their most recent common ancestor if a model of Brownian motion evolution is assumed). For example, if species 1 and 2 are sister species on the phylogeny and v_1 and v_2 are the lengths of the respective branches leading from their inferred common ancestor, the standardized evolutionary contrasts for this species pair under the assumption of Brownian motion character evolution are given by

$$E_{X_{12}} = \frac{X_1 - X_2}{\sqrt{v_1 + v_2}},$$

$$E_{Y_{12}} = \frac{Y_1 - Y_2}{\sqrt{v_1 + v_2}}. \tag{5}$$

Note that the numerator (i.e., the "unstandardized" contrast value) for each is a measure of the distance between the two species along each of the phenotypic axes, but these measures also incorporate the direction of subtraction because they can be either positive or negative. A phenotype is estimated for the node just below this pair as a weighted average of their phenotypes, the weights being the reciprocal of the branch lengths leading to each species (Felsenstein 1985). This method is continued down the tree, with some additional terms added to each subsequent branch length for estimating ancestral phenotypes (Felsenstein 1985), until a complete set of $n - 1$ standardized evolutionary contrasts are constructed from the phenotypic data for the n species and their hypothesized phylogeny. Constructing separate contrast sets for each phenotypic variable in the data set allows the evolution of covariation among individual traits to be examined as well as the evolutionary rates of individual characters (Díaz-Uriarte and Garland 1996; Rohlf 2001). Estimates of the phenotypes for all internal nodes (i.e., hypothetical ancestors) in the phylogeny can also be constructed (see Rohlf 2001).

However, for high-dimensional data sets such as that resulting from the spherical harmonic representation of morphological shape, the covariation among hundreds of characters is not as interesting as quantifying the overall rate of evolution of the structure. Remember that the numerator of a standardized evolutionary contrast is the phenotypic distance between two species. Thus, standardized evolutionary contrasts as metrics of the overall rate of evolution in the character set can be calculated using the distance among species' phenotypes on all characters simultaneously (Klingenberg and Ekau 1996). Note that this overall measure ignores information about the direction of evolution. Returning to our two-character example above, if X and Y are orthogonal characters, the standardized evolutionary contrast that quantifies the total rate of evolution in the phenotypes of 1 and 2, since their last common ancestor, is

$$M_{12} = \frac{\sqrt{(X_1 - X_2)^2 + (Y_1 - Y_2)^2}}{\sqrt{v_1 + v_2}}. \tag{6}$$

Note that like $E_{X_{12}}$ and $E_{Y_{12}}$, M_{12} is a rate of evolution (i.e., distance/time), but it is a rate of total change in all traits simultaneously. This concept of a multivariate contrast can be extended to any number of phenotypic traits by simply calculating the appropriate distance measure among the species—in this case Euclidian distance is used (Klingenberg and Ekau 1996). The rest of the algorithm for constructing standardized evolutionary contrasts is conducted exactly as in the typical analysis. The hypothetical phenotypes associated with ancestral nodes are calculated for each character, and then the distances between them are calculated to construct the multivariate contrasts. Euclidian distances can be calculated directly if the characters are orthogonal to one another, as with the spherical harmonics, because the spherical harmonic functions form an orthogonal basis for shape. Otherwise, some transformation of the data must be applied to construct a set of orthogonal characters from the original data (e.g., PCs). Multidimensional measures of evolutionary rates and evaluations of rate heterogeneity (e.g., Freckleton et al. 2002; O'Meara et al. 2006) can also be calculated using the same shift to distances in a multidimensional space.

By choosing the appropriate scaling of branch lengths, one can test various models of evolution (Martins and Garland 1991; Hansen and Martins 1996; Pagel 1997, 1999). Under the assumption of Brownian motion evolution, the variance in the phenotypic distance between two taxa should increase linearly with the time since their common ancestor (Felsenstein 1985). Thus, greater distances are expected between species that have been separated for longer periods of time, and a regression of the distance between species (i.e., the numerator) against the branch length estimates of time (i.e., denominator) in the evolutionary contrasts should give a line with a positive slope (cf. Garland et al. 1992). Additionally, regressing the standardized evolutionary contrast values against their denominators should show no relationship (Garland et al. 1992). In contrast, if character change is punctuated at the time of speciation, the distance between two species should be independent of the amount of time they have been separated, and so a regression of the distances between species against the branch length estimates of time in their associated evolutionary contrasts should show no relationship. Additionally, regressing the values of standardized evolutionary contrasts calculated by assuming all branch lengths are 1.0 against the associated branch lengths that are proportional to time should show no relationship, because the rate of phenotypic change estimated by the contrasts depends on the number of speciation events and not time. Obviously, these tests assume that the model of character evolution and rates of change under that model are homogeneous across the phylogeny.

A different approach to evaluating the evolutionary model most consistent with the character distribution is to estimate κ in a maximum likelihood framework (Pagel 1997, 1999; Freckleton et al. 2002). In this approach, each branch in the tree is raised to the power of κ; the κ value that maximizes the overall likelihood of the phenotypic data is found using standard search algorithms (Besset 2001). A value of $\kappa = 1.0$ is consistent with continuous character change under Brownian motion, and $\kappa = 0.0$ is consistent with punctuated change at the time of speciation. To search for κ, we used the multivariate distance between each species and the hypothetical phenotype for the root node of the phylogeny as the phenotypic metric to calculate the likelihood value for a given κ (see eq. [4] of Freckleton et al. 2002). The phylogenetic root phenotype and evolutionary rate estimates were recalculated for each κ value being evaluated.

Reconstructions used the *Enallagma* phylogeny presented in figure 1 (McPeek and Brown 2000; Turgeon et al. 2005). All polytomies were assumed to be hard polytomies (Purvis and Garland 1993; Rohlf 2001); for the evolutionary contrast analyses, we resolved polytomies in the *hageni* and *carunculatum* clades into dichotomous branches according to the resolution in the AFLP analyses of these two clades presented by Turgeon et al. (2005). Other polytomies in the phylogeny were arbitrarily resolved. All branch lengths in polytomies were set to 0 for all analyses (Purvis and Garland 1993; Rohlf 2001). We used the "censured" method of O'Meara et al. (2006) to estimate rates of character change and test for rate heterogeneity among the five major *Enallagma* clades for which we had more than one species (i.e., excluding the Southwest clade). All evolutionary analyses were performed by a program written in Java 1.5 (Sun Microsystems, Santa Clara, CA) and available from M. A. McPeek upon request.

Identical sets of analyses were performed on the right and left cerci. Because these structures are bilaterally symmetrical with one another, the results of these analyses were almost identical. Therefore, we present results only for the right cerci.

Results

Intraspecific Variation

The first 10 PCs extracted from the spherical harmonic coefficients of 41 *Enallagma hageni* individuals accounted for 78.2% of the total variation in cerci shape. The resulting PCs scores are complex numbers, but the imaginary parts of scores for the first 10 PCs were infinitesimal (on the order of 10^{-7}) relative to the real parts (on the order of 10^{0}–10^{-2}). Therefore, we consider that only the real parts of PC scores in this ordination maintain the lower di-

mensionality. Figure 3*a* illustrates the degree of overlap in cerci shape among the five populations for the first two PCs, and the remaining PCs show similar degrees of overlap among populations. The MANOVA of these 10 PCs detected no differences among the five populations from which these individuals were taken (Wilks's λ approximation, $F = 0.30$, df $= 40, 104$, $P > .50$).

Unlike shape, cerci size did vary significantly among populations (MANOVA of cerci length, surface area, and volume: $F = 4.30$, df $= 9, 85$, $P < .001$). These populations also differed in the measures of overall body size (MANOVA of head width, forewing length, and abdomen length: $F = 3.89$, df $= 9, 61$, $P < .001$), and the measures of cerci size were all positively correlated with the body size measures (Pearson correlations all with $P < .05$). Thus, cerci size differences reflect individual differences in overall body size, whereas shape appears to be invariant across populations.

Intraspecific shape variation was also much lower in general than differences among species. The intraspecific pairwise distances among *E. hageni* individuals in the spherical harmonic space averaged 0.14 ± 0.03 (mean $\pm$ 1 SD, $N = 595$). In contrast, the interspecific pairwise distances among the 41 individual specimens for each *Enallagma* species averaged 0.47 ± 0.14 (mean $\pm$ 1 SD, $N = 820$; fig. 3*b*). Only the distances between five species pairs overlapped with the intraspecific distribution (fig. 3*b*). These were all allopatric species pairs: the Asian *Enallagma circulatum* was close to both of the North American *Enallagma boreale* and *Enallagma laterale*; one pair has one species in Florida (*Enallagma cardenium*) and one in the Caribbean (*Enallagma coecum*); the species of one pair are on disjunct parts of the Atlantic coastal plain (*Enallagma pictum* and *Enallagma concisum*); and the last pair included the southwestern *Enallagma eiseni* and the North American *Enallagma basidens*.

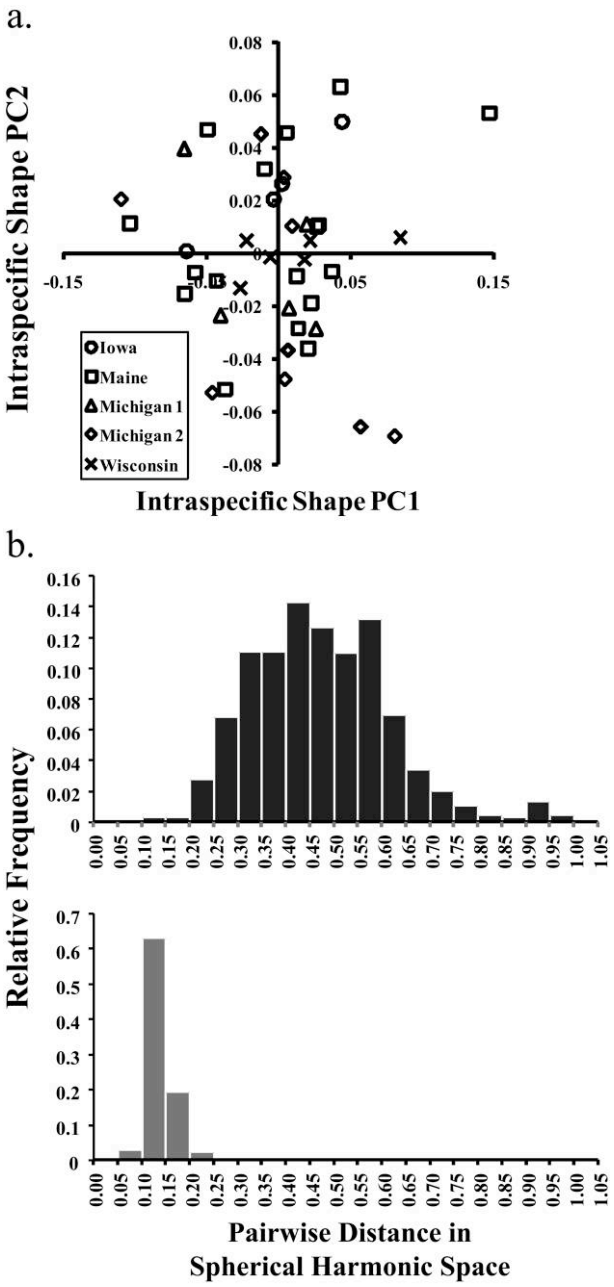


Figure 3: Results of the (*a*) ordination of the first two intraspecific shape principal component scores for individuals from five *Enallagma hageni* populations and (*b*) frequency histograms of the pairwise distances between the specimens of all species included in the interspecific analysis (*top*) and the pairwise distances between individuals of *E. hageni* in the five populations (*bottom*).

Tempo and Mode of Cerci Evolution

Because the intraspecific distances are generally much shorter than interspecific distances, and because cerci shape is independent of body size and does not vary among populations for *E. hageni*, we assume that the specimen included in the interspecific analyses for each species is characteristic of the cerci phenotype of that species (Harmon and Losos 2005). Also, because cerci size appears to change with overall body size, we do not present an interspecific analysis of size evolution.

To visualize the positions of species relative to one another in the high-dimensional spherical harmonic space, we again ordinated species based on their spherical harmonic coefficients using a PCs analysis of the complex spherical harmonic coefficients (extracted from the covariance matrix). Again, the imaginary parts of species scores for the first several PCs were infinitesimal (on the order of 10^{-7}) relative to the real parts (on the order of 10^{0}–10^{-2}). We consider only the real parts of the PC scores

in this ordination to maintain the dimensionality at three. The first three PC axes accounted for 71.5% of the variation among the species. Lower frequencies in the spherical harmonics account for most of the variation along these PC axes (fig. 4).

Patterns of PC loadings are not directly interpretable as differences in specific shape features, but back projection of the spherical harmonic model along the PC axes allowed us to interpret shape differences characterized by each axis. All *Enallagma* cerci can be interpreted as variations on the basic shape of a two-tined fork (identified by landmarks *1–4* for *Enallagma ebrium* in fig. 2*b*), with differences among species being variations on the lengths, thicknesses, and angles of the tines. The PC1 quantified a shift from the superior tine being long relative to the inferior tine and the inferior tine projecting down (more negative val-

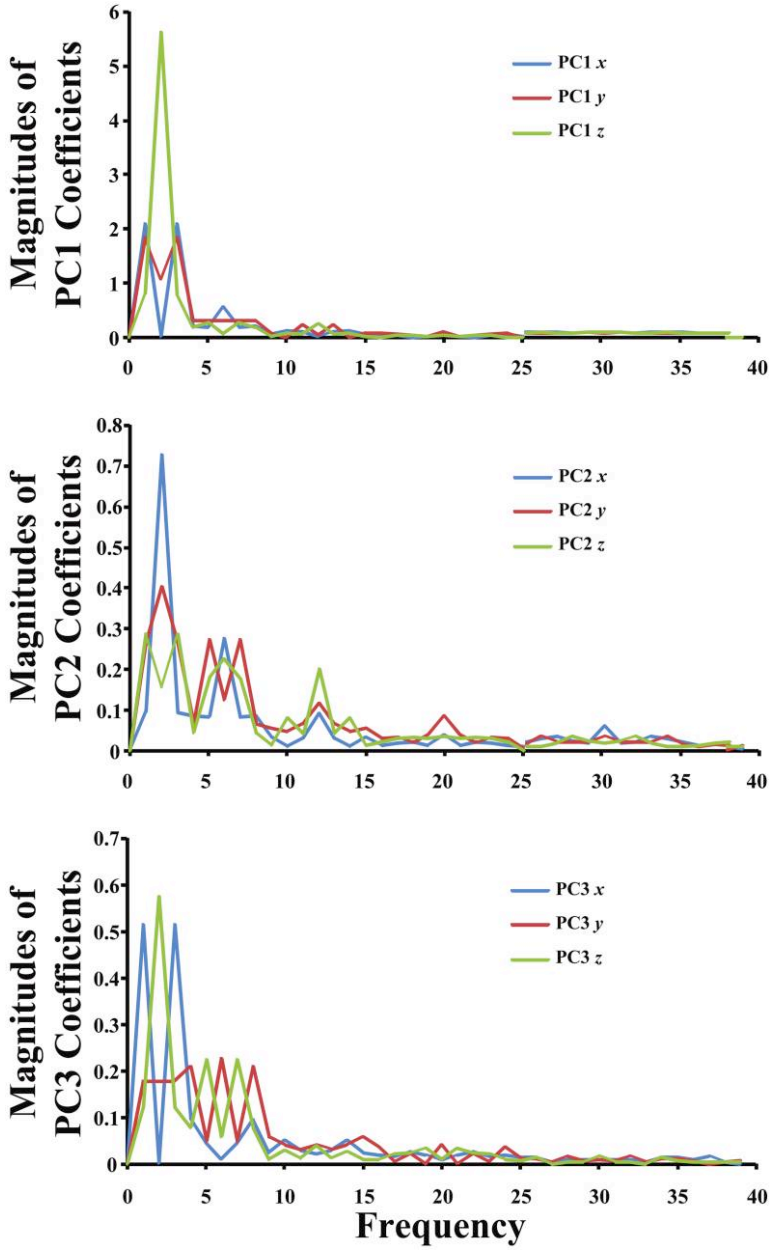


Figure 4: Magnitudes of the principal component (PC) coefficients of the first 40 spherical harmonic coefficients for each of the *x*, *y*, and *z* dimensions for the first three PCs of shape.

ues) to the superior and inferior tines being small and relatively indistinct from one another (more positive values; fig. 5). The PC2 quantified a shift from cerci with a long superior tine and a very short and ventral projecting inferior tine (more negative values) to cerci with a short superior tine and a long but tapered inferior tine (fig. 5). The PC3 quantified a shift from a cercus with both tines relatively small (more negative values) to a cercus with a broad inferior tine (more positive values; fig. 5). The PC4 accounted for only 4.0% of the variation. The clades were significantly displaced from one another in this 3-D reduced space (MANOVA on the first three PCs: Wilk's λ approximation, $F = 6.46$, df $= 15, 92$, $P < .001$).

While the clades differ in average shape today, multivariate standardized evolutionary contrasts analyses indicated that they developed those patterns by similar tempos and modes of evolution. First, no relationship was apparent between unstandardized evolutionary contrast values (i.e., the distances between the pairs of species) and the associated branch lengths that are proportional to time ($r = 0.13$, df $= 35$, $P > .45$; fig. 6*a*). Also, no relationship with branch length was apparent when multivariate contrasts were standardized by the number of speciation events, whereas a strong negative power relationship was apparent when they were standardized by branch lengths (fig. 6*b*). Finally, the maximum likelihood estimate of κ was 4.3×10^{-9} ($\log L = 21.2$). These results are all consistent with the evolution of cerci shape following a model of punctuated change at the time of speciation.

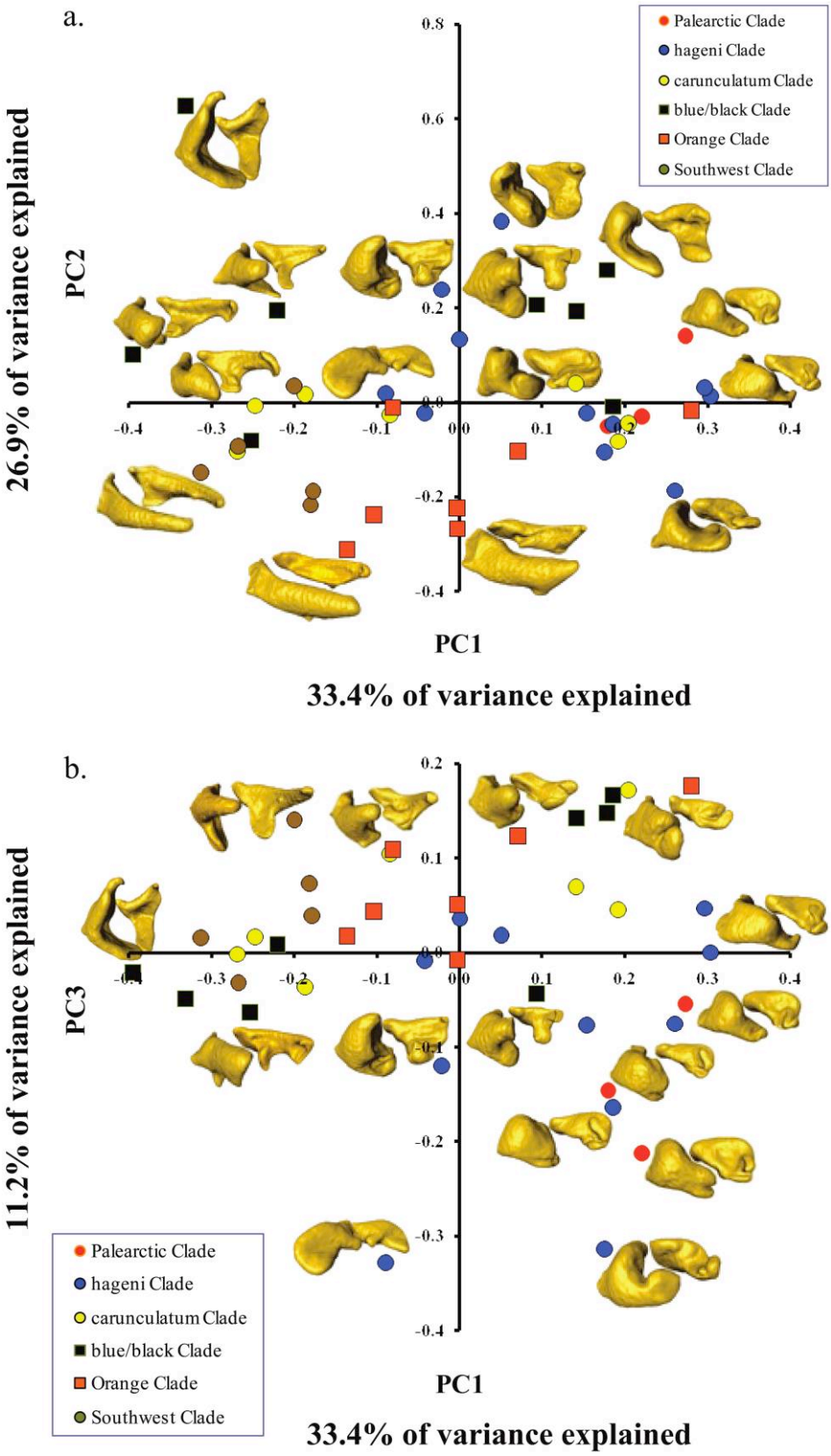
Assuming punctuated change, we also tested for evolutionary rate heterogeneity among the five clades using the multivariate shape contrasts (table 1). Comparisons using traditional ANOVA and the multivariate standardized evolutionary contrasts as the raw data indicated no differences among the five clades included in the analyses ($F = 2.03$, df $= 4, 27$, $P > .10$). Also, a model assuming the same evolutionary rate of shape change across all clades was favored over a model assuming a separate rate for each clade (likelihood ratio test: $\chi^2 = 3.31$, df $= 4$, $P > .45$; also, the model with a single rate for all clades had an Akaike Information Criterion (AIC_c) $= 4.61$, and the model with separate rates for each clade had $AIC_c = 17.9$).

Estimates for the real and imaginary parts of the spherical harmonic coefficients were used to reconstruct the phenotypes of the hypothetical ancestors throughout the *Enallagma* phylogeny (fig. 7); a zip file available in the online edition of the *American Naturalist* presents interactive 3-D views of all the species and ancestral models. The hypothetical phenotypes of the last common ancestors of the Palearctic and *hageni* clades are very similar, with each being broad relative to length and having very indistinct and short superior and inferior tines. The hypothetical phenotype of the orange clade ancestor has a relatively long and narrow cercus, because the inferior tine is very short, downturned, and quite broad (almost the entire ventral surface can be interpreted as the tip of the inferior tine). The hypothetical phenotype of the *carunculatum* and blue/black clades have broad superior tines and more narrow and ventrally pointing inferior tines. Interestingly, the last common ancestors of the two primary clades in the genus have very similar hypothetical phenotypes that are similar to the *carunculatum* and blue/black clade ancestors. We take this as evidence of no biases or trends in cerci shape evolution. As a result, the hypothetical phenotype for the root node of the phylogeny also has a relatively broad superior tine and a more narrow and ventrally pointing inferior tine.

Discussion

Traits associated with generating and maintaining reproductive isolation through species mate recognition are expected to change at the time of speciation (i.e., when reproductive isolation is generated) but to change little in intervening periods (Templeton 1979; Paterson 1993). Rapid change at the time of speciation is obvious by definition, because it is change in these traits that generates reproductive isolation. Moreover, many mechanisms may cause rapid diversification in such traits, for example, founder events (Templeton 1979; Carson and Templeton 1984) or species interactions (Payne and Krakauer 1997; Gavrilets 2004; McPeek and Gavrilets 2006). However, once lineages have differentiated sufficiently and individuals can unambiguously discriminate potential mates as conspecifics or heterospecifics, the rate of character change should slow appreciably (McPeek and Gavrilets 2006). In fact, interspecific mate choice and recognition is expected to impose strong stabilizing selection on these characters in both males and females, since extreme phenotypes would be less likely to obtain viable matings (Lande 1981, 1982; Sved 1981*a*, 1981*b*; Kirkpatrick 1982). This scenario of selection on traits defining reproductive isolation thus predicts that patterns of interspecific character evolution should follow a model of punctuated change that should be consistent with the number of speciation events in the clade.

Our analyses of the tempo and mode of cerci shape evolution strongly support this model of punctuated change at the time of speciation as constructing the extant diversity in cerci types among the *Enallagma* damselflies. Multivariate evolutionary contrasts standardized by branch lengths all set to 1 showed no relationship with genetic distances between the pairs of species involved (fig. 6). This result would obtain if the degree of phenotypic differentiation generated at the time of speciation were


a.
Palearctic Clade
hageni Clade
carunculatum Clade
blue/black Clade
Orange Clade
Southwest Clade
26.9% of variance explained
PC2
PC1
33.4% of variance explained
b.
11.2% of variance explained
PC3
PC1
33.4% of variance explained

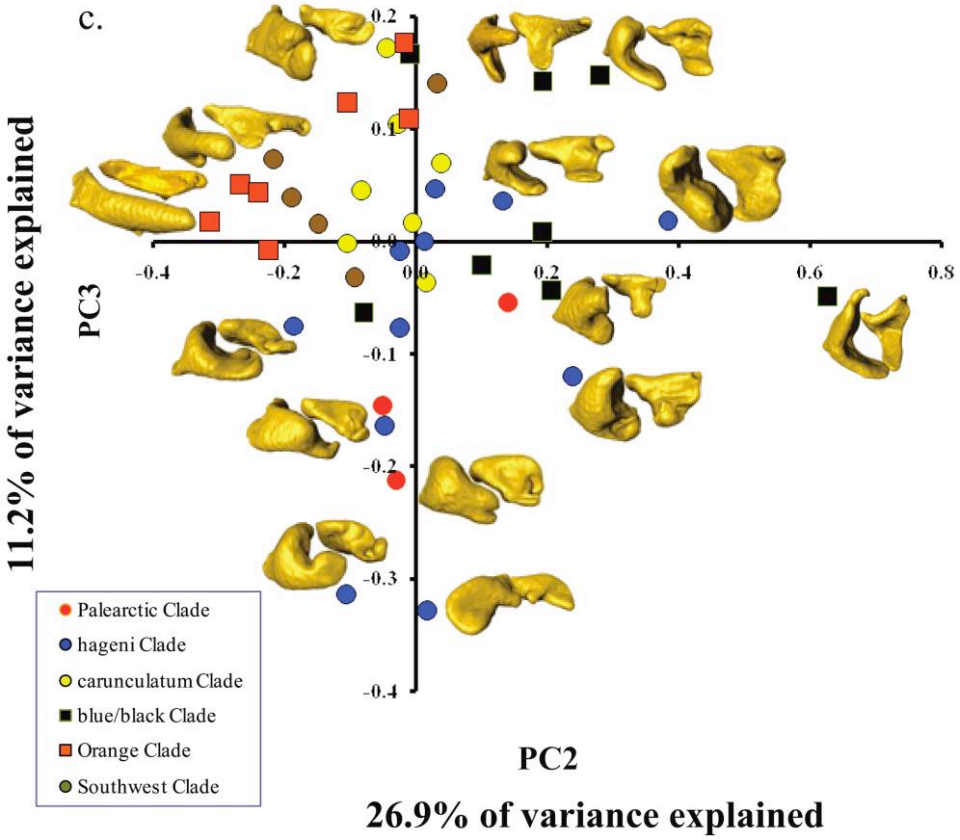


Figure 5: Principal component (PC) ordination of all *Enallagma* species based on the spherical harmonic coefficients derived from size-standardized cerci. The loadings for these three PCs are given in figure 4. *a*, *b*, and *c* illustrate the 3-D relationships among the species in a series of 2-D plots. Each point is the position of the right cercus for a species. The points are color coded to identify the clade membership of the species (*inset*). Visual representations of the spherical harmonic models for selected species are placed next to their corresponding points to identify the shape variation that is captured along the axes of the ordination. The amount of variation explained by each PC is given in the axes labels.

relatively constant across the entire phylogeny. In contrast, multivariate evolutionary contrasts standardized by branch lengths based on genetic distances, and thus assumed proportional to the time species have been separated, showed much larger values for contrasts involving shorter branches: this is the relationship expected if a constant amount of phenotypic difference is divided by increasingly larger numbers for branch lengths (fig. 6*b* for Brownian motion). In addition, the maximum likelihood estimate of κ being on the order of 10^{-9} also argues strongly for the punctuated model of character change. Rate homogeneity across the entire phylogeny was also the most likely model of cerci shape change (table 1). Moreover, no intraspecific differentiation between widely spaced populations was evident for at least one species.

These results are even more striking when the relative ages of the various *Enallagma* clades are considered. The radiations of the Palearctic, *hageni*, and *carunculatum* progenitors that resulted in 21 extant species all appear to have occurred within the last 250,000 years (Turgeon et al. 2005). The orange clade is somewhat older at ~2 million years. However, the blue/black clade extends back to nearly the time of the last common ancestor of the entire genus at ~13 million years, with some species being apparently very old (fig. 1). With Brownian motion evolution, trait variance among species within a clade is expected to increase linearly with time (Felsenstein 1985). Thus, with continuous character change, the variance among species in the blue/black clade is expected to be approximately 52 times greater than in any of the three recently radiating clades. Despite these vast differences in the crown group ages of the various clades, the volumes of phenotype space occupied by the six clades are all very similar (e.g., fig. 5), as expected under a punctuated model of evolution. These results taken together all argue that throughout the history of the *Enallagma*, cerci shape has rapidly differentiated at

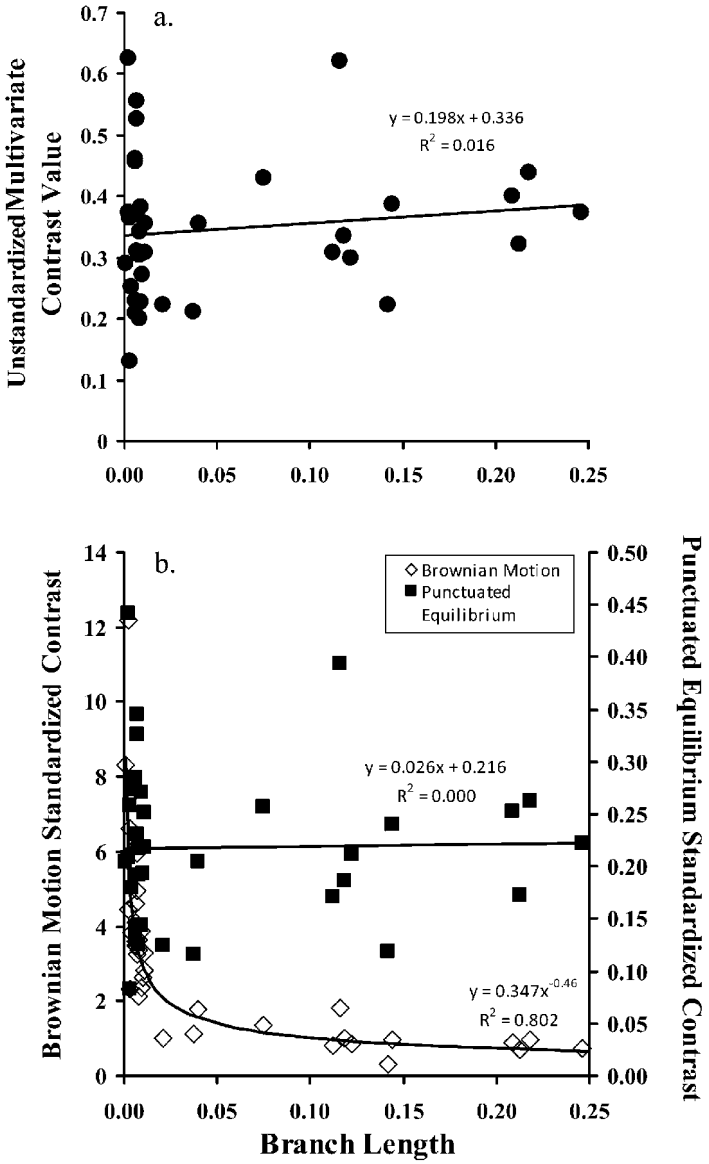


Figure 6: Results of evolutionary contrasts analyses. *a*, The unstandardized multivariate evolutionary contrast values (i.e., the Euclidian distance between the two taxa in the contrast) regressed against the sum of the branch lengths for the respective contrasts. *b*, Standardized multivariate evolutionary contrast values calculated according to the Brownian motion model (*diamonds*) and the punctuated model (*squares*) of evolution are regressed against the sum of the branch lengths associated with the corresponding contrasts. Note that the branch lengths used to calculate the standardized contrast for the Brownian motion model are the same as those for each point on the *X*-axis. All branch lengths were set to 1.0 to calculate standardized contrasts under the punctuated model, but these contrasts are regressed here against the branch lengths used to calculate the Brownian motion model.

the time of speciation by a relatively constant amount and has changed little if at all until the next speciation event in a lineage.

These are the evolutionary dynamics expected for a structure that is part of a species mate recognition system (Templeton 1979; Paterson 1993). *Enallagma* males are very promiscuous and will attempt to mate with most *Enallagma* females regardless of species (Paulson 1974; Robertson and Paterson 1982; Fincke et al. 2007). Experiments suggest that females use tactile cues caused by the contact of the cerci with the female's mesostigmal thoracic plates to identify suitable mates. Conspecific males with experimentally altered cerci are rejected by females just as heterospecific males are rejected, but females mate with

Table 1: Evolutionary rate estimates for the cerci shape analyses

Clade	Species richness	Shape multivariate $\hat{\sigma}^2$
Palearctic	3	.029
"*hageni*"	10	.056
"*carunculatum*"	7	.035
Blue/black	8	.078
Orange	7	.030
Entire genus	37	.052

Note: Rate estimates derived using the censured method of O'Meara et al. (2006). The "Shape multivariate" column gives rate estimates derived for the multivariate contrasts of the spherical harmonic coefficients of shape. The first five rows of the table give the rate estimates assuming that each of the five major clades included in the analysis have separate rates. The last row ("Entire genus") is the rate estimate derived assuming that all clades have the same evolutionary rate. Rate estimates are calculated using equation (2) of O'Meara et al. (2006).

conspecific males with intact cerci (Robertson and Paterson 1982). The evolutionary dynamics of cerci shape are consistent with females using these tactile cues primarily to categorize males as conspecifics or heterospecifics and not as a more subtle indicator of male quality, as would be expected in various forms of sexual selection. Also, these dynamics are not consistent with an evolutionary arms race in which the shape of the female mesostigmal plates are evolving as defenses against being grasped and controlled by the male cerci (cf. Arnqvist and Rowe 2002*a*, 2002*b*). We are currently repeating these types of analyses with the female mesostigmal plates and developing methods to quantify the fit between these male and female structures in conspecific and heterospecific matings to digitally estimate what females feel during matings with different males. We predict that the female plates will show the same evolutionary dynamics as male cerci and that these structures will display all the characteristics expected in a lock-and-key species mate recognition system (Shapiro and Porter 1989; Arnqvist 1997), even though these are not genitalia.

Enallagma lacks the conspicuous wing markings and other features on which sexual selection via female choice has been shown to operate in other odonates (e.g., Koenig 1991; Grether 1996; Svensson et al. 2006). However, the stabilizing selection imposed by female mate choice on cerci shape may indirectly impose some degree of stabilizing sexual selection on male body size. If each female has a mate preference function (Lande 1981; Sved 1981*a*, 1981*b*; Kirkpatrick 1982) that defines an optimal tactile sensation to accept males as mates, males that are farther from a female's tactile optimum will be less likely to mate. Heterospecific males that possess very different cerci from conspecifics will generate tactile sensations that are far from her preference optimum and thus will be quickly rejected—this is the basis of species recognition. In addition, the tactile sensations that any particular female feels when she is grasped by conspecific males of various sizes will also depend on the fit between the males' cerci and her thoracic plates. Because cerci size scales with overall body size (at least for *Enallagma hageni*), males of more extreme sizes may be less likely to mate with a particular female because the sizes of their cerci also generate tactile sensations farther from her preference optimum. Consistent with this prediction, we have quantified stabilizing sexual selection for male size in a population of *Enallagma aspersum* (D. Steele and M. A. McPeek, unpublished article; see also Stoks 2000 for another example of stabilizing sexual selection on body size in a damselfly species). Intraspecific sexual selection may thus play some role in the evolutionary dynamics of cerci size and thus indirectly on overall body size.

The literature is quite mixed about the relative importance of various mechanisms in shaping the evolution of genitalia and other sexual structures such as the cerci and mesostigmal plates of damselflies. In arthropods, a lock-and-key mechanism of genital fit (Dufour 1844) is thought to enforce reproductive isolation in many taxa because of the taxonomic utility of genital morphologies in these groups. However, the hypothesis has fared poorly in empirical tests (e.g., Eberhard 2005; Mutanen et al. 2006; see Shapiro and Porter 1989 for a general review). Fisherian runaway sexual selection is expected to produce positive allometric scalings of sexual parts, but taxa show a wide diversity of allometric scaling patterns of sexual traits (reviewed in Hosken and Stockley 2003). However, the allometric scaling expected for sexual traits may be more complicated than simple models would predict (Bonduriansky 2007), and emergent intraspecific allometry may not even coincide with those predicted from the form of selection (e.g., directional versus stabilizing) acting on a structure (Bertin and Fairbairn 2007). Other methods of analysis have also identified sexual selection on genital morphology, but again, not universally (e.g., Arnqvist 1998; Polihronakis 2006). Similarly, sexually antagonistic coevolution of male and female sexual traits shapes interspecific variation in some taxa (Arnqvist and Rowe 2002*a*, 2002*b*) but not others (e.g., Eberhard 2004, 2005).

If *Enallagma* cerci evolution is driven primarily by species mate recognition and not by sexual selection or sexual antagonism, why does cerci shape differentiate to create new species at all? Often, such mechanisms of premating reproductive isolation are thought to evolve to reduce the costs of producing less viable or less fertile hybrids from mating with individuals in lineages that are already partially differentiated from the species in question (Kelly and Noor 1996; Kirkpatrick and Servedio 1999; Kirkpatrick 2000, 2001). This "reinforcement" of preexisting postmating differentiation has probably not been important

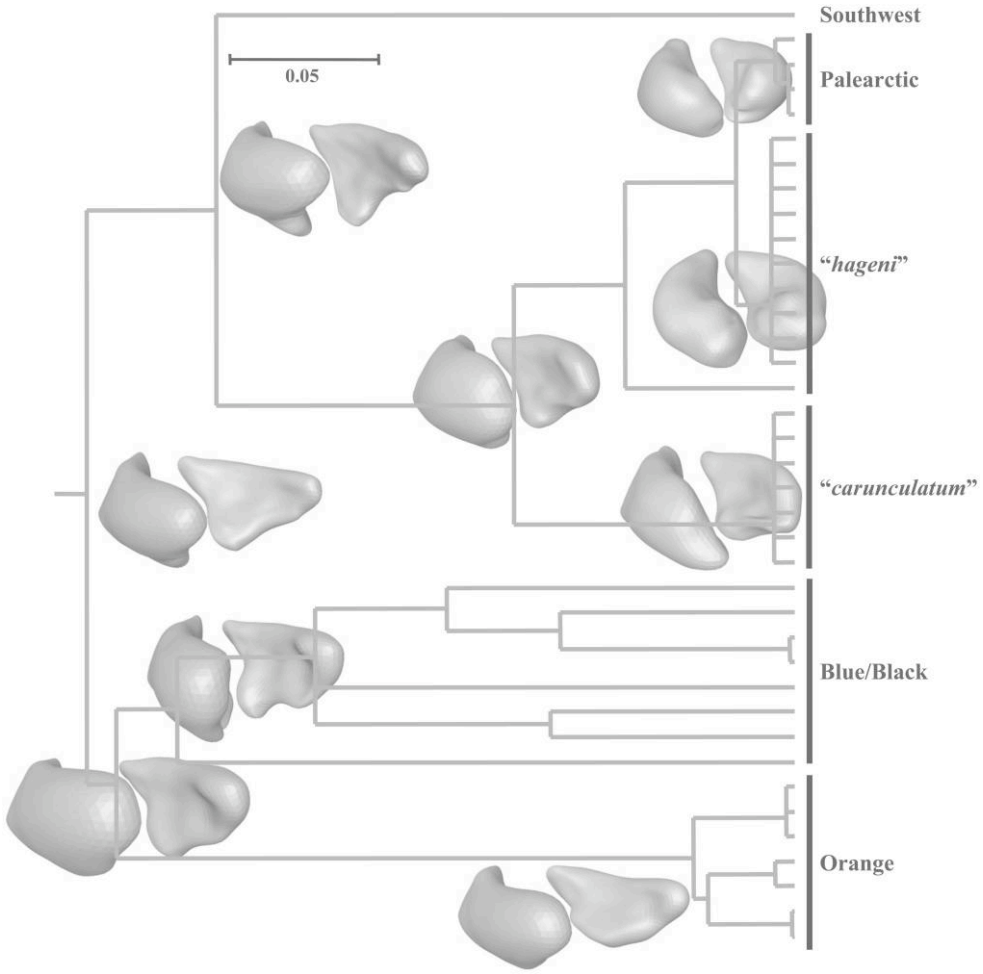


Figure 7: Ancestral cerci shapes for the various clade ancestors derived from the ancestral reconstructions of spherical harmonic coefficients in the evolutionary contrasts analyses assuming a punctuated model of character evolution. Left and right cerci are shown for each ancestor, and cerci pairs are placed either under or immediately adjacent to the corresponding node of the phylogeny. The view of each pair is at an oblique angle from the rear, with the left cercus to the left (the viewer can see the outer surface of the left cercus) and the right cercus to the right (the viewer can see the inner surface of the right cercus). Interactive 3-D views of all species and ancestral models can be found in a zip file available in the online edition of the *American Naturalist*.

in *Enallagma*. For example, many of the species within each of the three recent radiations share identical 1,000-bp haplotypes spanning the cytochrome oxidase I and II genes in the mitochondrial genome (e.g., some haplotypes are found in up to five species), and overall species show little or no interspecific differentiation in mtDNA sequences within radiations, but almost all are differentiated at much more rapidly evolving AFLP loci (Turgeon et al. 2005). The facts that (1) mitochondrial genes are some of the most rapidly evolving genes in animals and (2) many of the species within each radiation are difficult to distinguish except for their cerci and mesostigmal plates suggest that species within each radiation may be genetically quite compatible with one another and would show no degree of hybrid inviability or infertility were they to interbreed. Thus, these species probably have had no impetus to evolve

prereproductive isolating mechanisms to reduce or prevent hybridization with others during the radiation.

Cerci differentiation also cannot be completely explained as a correlated by-product of ecological differentiation (Rice and Hostern 1993; Gavrilets 2004). In the two recent North American radiations, four different speciation events appear to be the product of ecological speciation. Three of these (one in the *hageni* clade and two in the *carunculatum* clade) were independent habitat shifts in which lineages invaded and adapted to living with dragonfly predators in fishless ponds, a new habitat for the *Enallagma* because the rest of the species in the genus are adapted to living only in ponds and lakes with fish (McPeek 1990, 1995*b*, 1998, 1999; McPeek et al. 1996; McPeek and Brown 2000; Turgeon et al. 2005; Stoks and McPeek 2006). The other produced a lineage that is now endemic to the Atlantic coastal plain; presumably, this lineage adapted to ecological conditions specific to coastal plain lakes with fish (e.g., perhaps physiological adaptation to lake water chemistry under oceanic influences; Turgeon et al. 2005).

However, the remaining 11 speciation events in these two clades have no clear ecological explanations. For example, the lineage that shifted onto the coastal plain subsequently underwent three additional branching events to produce four endemic species, all of which are phenotypically identical as larvae and adults, except for their cerci (Stoks et al. 2003; McPeek 2004; Stoks and McPeek 2006), and all are locally sympatric where their ranges overlap (M. A. McPeek, unpublished data). Similarly, one of the lineages that adapted to dragonfly ponds subsequently underwent a second speciation event after the shift to produce two species (*Enallagma annexum* and *Enallagma boreale*) that are phenotypically identical, again except for their cerci (Stoks et al. 2003; McPeek 2004; Stoks and McPeek 2006), and that can now be found together in these ponds from the Atlantic to the Pacific oceans (Stoks et al. 2005; M. A. McPeek, unpublished data). Finally, the remaining nine species in the two clades are also phenotypically very similar (McPeek 2004; Stoks and McPeek 2006), have large and overlapping distributions (Westfall and May 2006), and can be found locally sympatric in fish ponds and lakes across the continent (M. A. McPeek, unpublished data). Thus, the majority of the speciation events in these two radiations seems to have been caused directly by the coordinated differentiation of male cerci and female thoracic plates.

Biogeographic and phylogeographic data suggest that the mechanisms driving differentiation in the species mate recognition systems in these radiations were associated with the periodic Pleistocene glacial advances and retreats. Most species in the two recent North American radiations currently have ranges that would have been completely under ice at the last glacial maximum (Donnelly 2004). Most species in both radiations also bear striking phylogeographic signatures of either range fragmentations and expansions (Turgeon et al. 2005). Fragmentation of species' ranges caused by glacial advance may have caused local genetic bottlenecks that generated differentiation in the cerci and thoracic plate morphologies among the range fragments to create new species (e.g., Templeton 1979; Lande 1981; Carson and Templeton 1984). Rapid range expansions as glaciers retreated could also have generated genetic drift in these characters to make new species (Carson and Templeton 1984; Gavrilets and Boake 1998; Regan et al. 2003).

In addition, we have identified two areas in North America where unidirectional mitochondrial hybridization occurred briefly in the recent past between members of the two clades (New England and northern California; Turgeon et al. 2005). Unidirectional mitochondrial hybridization is frequently seen when females of one species are locally rare and cannot find conspecific males, and they eventually acquiesce to mating with males of the locally common species (Wirtz 1999; Randler 2002). Such social situations would have been quite common across the entire continent as species colonized newly formed lakes in newly deglaciated areas; because local population sizes can be on the order of 10^5–10^7 at a given lake, females of later-arriving species to a given lake would have experienced primarily heterospecific males until their population numbers increased substantially. These social conditions can also impose strong selection on female mating preferences to differentiate rapidly among populations that are interacting with different sets of species (Hoskin et al. 2005; Pfennig and Pfennig 2005; McPeek and Gavrilets 2006). Recolonizing deglaciated areas may have thus directly imposed substantial selection for differentiation in species mate recognition.

Clearly, no single evolutionary force shapes the evolution of sexual traits across all animals, and in fact, sexual structures in many taxa may simultaneously experience selection pressures from all these evolutionary forces to varying degrees (Arnqvist 1997; Hosken and Stockley 2003; Cordero and Eberhard 2005). Theoretical representations are beginning to understand intraspecific mate choice and species recognition as lying along a continuum (Boake et al. 1997; Castellano and Cermelli 2006; Phelps et al. 2006). Because the evolution of species recognition is one substantial way that reproductive isolation is generated among lineages, the evolution of characters shaping intraspecific and interspecific mate choice are fundamental to understanding the generation and maintenance of species diversity patterns in many groups.

Acknowledgments

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APPENDIX

Table A1: Locality and collection date for the *Enallagma* specimens included in the interspecific analysis of cerci shape

Genus and species	Location	Date collected
Enallagma anna	Bloody Creek and County Line Road, Loup Co., Nebraska	July 20, 2005
Enallagma annexum	Sylvester Pond, Norwich, Vermont	June 12, 2007
Enallagma antennatum	Chemung River, Bottcher's Landing, Big Flats, Chemung Co., New York	July 3, 2004
Enallagma aspersum	Pond "8," Enfield Corner's Road, Enfield, Tompkins Co., New York	July 1, 2003
Enallagma basidens	San Sabo River, 0.7 miles. northeast of Fort McKavitte, Menard Co., Texas	July 15, 2001
Enallagma boreale	Colton, 3 miles southwest of Orebed Road, St. Lawrence Co., New York	June 5, 2005
Enallagma cardenium	Chicken Branch, County Road 154, Tallahassee, Leon Co., Florida	April 17, 2006
Enallagma carunculatum	Milburn Diversion Dam State Wildlife Management Area, 2 miles northwest of Milburn, Blaine Co., Nebraska	July 18, 2005
Enallagma circulatum	Lake Aliger, Kuril Island, Kunashir, Russia	August 26, 1996
Enallagma civile	Lewis Ocean Bay Heritage Preserve, 9 miles east-southeast of Conway, Horry Co., South Carolina	August 30, 2005
Enallagma clausum	Bear River Migratory Bird Refuge, 13 miles west of Brigham City, Box Elder Co., Utah	June 27, 1905
Enallagma coecum	Basse-Terra Riviere de Beugendre northeast of Marigot, Guadeloupe	January 29, 2006
Enallagma concisum	Marsh 1.5 miles North of entrance to Blackwater River State Park, Santa Rosa Co., Florida	April 12, 2004
Enallagma cyathigerum	Zwart Water, Belgium	July 26, 1999
Enallagma daeckii	Blackwater River State Park, Santa Rosa Co., Florida	April 13, 2004
Enallagma davisi	Dog Lake, Tallahassee, Florida	March 26, 1995
Enallagma divagans	Adams Spring Branch and Indian Fork Road, Santa Rosa Co., Florida	April 12, 2004
Enallagma doubledayi	Lewis Ocean Bay Heritage Preserve, 9 miles east-southeast Conway, Horry Co., South Carolina	August 29, 2005
Enallagma dubium	Trout Pond, State Road 373, Tallahassee, Florida	May 14, 1995
Enallagma durum	Canal on Ferry Road, Old Sambrook, Connecticut	July 7, 2001
Enallagma ebrium	Colton, 3 miles southwest of Orebed Road, St. Lawrence Co., New York	June 5, 2005
Enallagma eiseni	Sur, Pond at Rancho San Enrique, 51 km east of Villa Insurgentes, Baja California, Mexico	October 4, 1984
Enallagma exsulans	Genesee River and Cronk Hill Road, Hume, Allegany Co., New York	July 15, 2003
Enallagma geminatum	Stone Mill Pond, Madison Co., New York	July 14, 1999
Enallagma hageni	Palmatier Lake, Hastings, Michigan	June 17, 2001
Enallagma laterale	Perley Pond, Sebago, Maine	June 24, 1995
Enallagma minusculum	Norton Pond, 4 miles east of Brownville Junction, Piscataquis Co., Maine	June 26, 1987
Enallagma novaehispaniae	Domatila new Rio Dorado, Granada Department, Nicaragua	August 27, 2003
Enallagma pallidum	Trout Pond, State Road 373, Tallahassee, Florida	May 14, 1995
Enallagma pictum	Whitesbog, Lebanon State Forest, Burlington Co., New Jersey	June 8, 1991
Enallagma pollutum	Francis S. Taylor Wildlife Management Area at Route 41, 12 miles west of Route 997, Dade Co., Florida	April 3, 2005
Enallagma recurvatum	Cedar Lake, State Road 347 and 550 Spur, Bellplain State Forest, Cape May Co., New Jersey	May 22, 1995
Enallagma risi	Manzhouli, China	July 23, 1999

Table A1 (*Continued*)

Genus and species	Location	Date collected
Enallagma semicirculare	Los Tuxtlas Biological Station, 30 km north-northeast of Catemaco, Laguna Azul, Veracruz, Mexico	August 27, 1988
Enallagma signatum	Roger Nature Center, Sherbourne, Chenango Co., New York	July 13, 1999
Enallagma sulcatum	Lake Alto, Waldo, Florida	April 18, 1990
Enallagma traviatum	Pond "18," County Road 6, Hector, Schyler Co., New York	July 1, 2002
Enallagma vernale	McDaniels Marsh, Enfield, New Hampshire	June 11, 2007
Enallagma weewa	Burnt Mill Creek, US 27 east of Tallahassee, Florida	May 14, 1995

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