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THE CORRELATED EVOLUTION OF THREE-DIMENSIONAL REPRODUCTIVE STRUCTURES BETWEEN MALE AND FEMALE DAMSELFLIES

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For many taxa, species are defined by the morphologies of reproductive structures. In many odonates, these structures are the cerci of males (used to hold females during mating) and the thoracic plates of females where the male cerci contact the females' bodies. A previous study showed that the shapes of cerci of *Enallagma* males (Zygoptera: Coenagrionidae) are best explained by an evolutionary model of punctuated change at the time of speciation, with a homogeneous rate of change across the entire phylogeny of the genus. In the present study, we examine the evolution of shape change in the corresponding female plates. We found that, like male cerci, the shapes of *Enallagma* female thoracic plates could best be explained by an evolutionary model of punctuated change at the time of speciation, with a homogeneous rate of change across the clade. Moreover, the evolutionary contrasts quantifying the rates of change in male cerci and female thoracic plates were positively related across the history of the clade, demonstrating that these male and female structures evolve in a correlated fashion. This pattern of evolution suggests that these structures are primary signals of species identity during mating.

KEY WORDS: **Mate recognition, mating structures, morphological evolution, punctuated change, reproductive isolation, spherical harmonics.**

The boundaries separating biological species are defined by the mating compatibilities and preferences of males and females. If a female cannot or will not mate to produce viable offspring with a particular male, these two individuals are—according to the Biological Species Concept—in effect different species (Mayr 1942). The reasons for these reproductive incompatibilities are manifold (Howard and Berlocher 1998; Coyne and Orr 2004). Gametes may be unable to fuse to produce a zygote (e.g., Swanson and Vacquier 2002; Springer and Crespi 2007), or the resulting zygote may be less viable or inviable (Noor and Feder 2006). In contrast, mate selection preferences of the female or male may prevent the mating from occurring in the first place (Dobzhansky 1937, 1940; Mayr 1942; Templeton 1979; Paterson 1993; Boake et al. 1997). Because these are the proximate mechanisms defining species boundaries, understanding the evolution of these various reproductive isolating mechanisms across taxa provide direct insights into the processes generating biological diversity.

In many arthropod groups, morphological features of genitalia and other reproductive structures are thought to define reproductive compatibility. Species are generally defined on the basis of these structures, and this is taken as evidence for the correlated evolution of these structures between males and females (Eberhard 1985; Huber 2003). The lock-and-key hypothesis is probably the best-known proposal for how preinsemination mating preferences are enforced by the morphological fit of male and female structures (Dufour 1844; Shapiro and Porter 1989; Arnqvist 1997). If such morphological compatibility enforces reproductive compatibility to define species boundaries, reproductive structures should show a characteristic pattern of evolution over the history of a clade. First, reproductive structures in each sex should change rapidly at or around the time of speciation because change in these characters defines new species, but these characters should change little between speciation events because of stabilizing selection for species' mate recognition (Eberhard 1985; Paterson 1993; Arnqvist 1997; Hosken and Stockley 2003). Also, stabilizing selection for mate recognition should favor large morphological differences between species relative to intraspecific differences (Gavrilets 2000; McPeek and Gavrilets 2006). Finally, male and female structures should show correlated patterns of evolutionary change.

Previously, we have shown that evolutionary change in the shapes of cerci of male *Enallagma* damselflies is completely consistent with these hypothesized evolutionary dynamics. Cerci are the male reproductive structures used to grasp females during mating. Experimental results have shown that females use the tactile cues from this grasping to determine the species identity of the male (Paulson 1974; Robertson and Paterson 1982). The best evolutionary model to explain cerci shape change over the phylogenetic hypothesis for *Enallagma* was one of punctuated change at the time of speciation, and a homogeneous rate of change across the phylogeny (McPeek et al. 2008).

The male cerci contact plates on the anteriodorsal surface of the mesothorax of females when males grasp females for mating (Fig. 1). Like the male cerci, these female thoracic plates are the diagnostic structures used to identify females to species (Westfall and May 2006). Damselfly females also use these structures to identify potential mates as conspecifics or heterospecifics: female plates are lined with setae that presumably define sensory cues obtained by females when grasped by the cerci of conspecific and heterospecific males (Paulson 1974; Robertson and Paterson 1982). Thus, the shapes of cerci should define the tactile cues a female obtains when grasped by males with different cerci shapes. Typically, a female will not mate with a heterospecific male, and he will release her in < 2 min (M. A. McPeek, pers. obs.). Females also refuse conspecific males with altered cerci, as they do heterospecific males (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 "*caruncul atum*" clades using AFLP loci found evidence for hybridization between only two pairs of species (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. We hypothesized that the evolution of female plate shape should mirror the results for male cerci evolution, namely change in plate shape should best fit a model of punctuated change at the time of speciation with homogeneous rates of change across the phylogeny. In addition, the shape of male cerci and female thoracic plates should change in a correlated evolutionary fashion across the phylogeny.

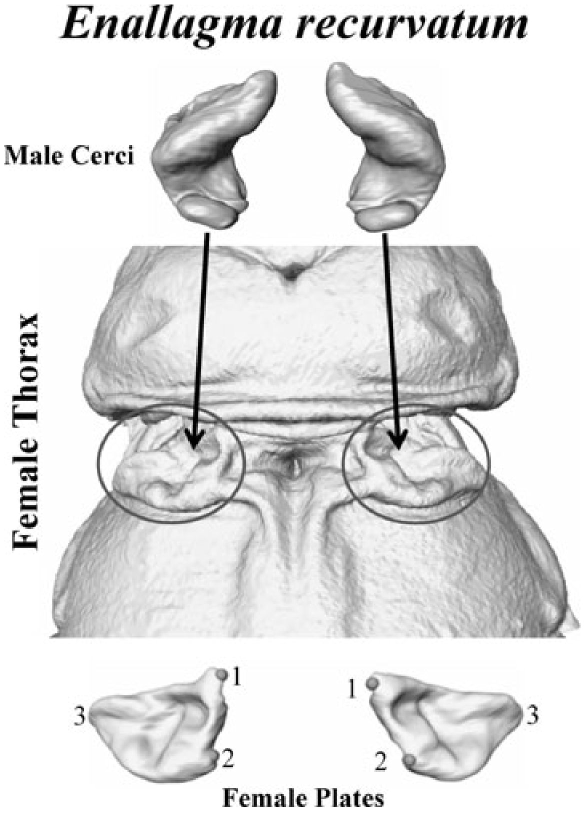

Figure 1. Computer models from computer tomography scans of the male cerci (the top two structures) and the female thoracic plates for *Enallagma recurvatum*. The male cerci are the two structures at the top of the image, and the female thoracic plates lie on the dorsoanterior surface of the mesothorax (circled with blue ovals). The arrows show where the male cerci contact and grip the female plates. At the bottom of the figure, the female plates have been segmented out of the rest of the thorax. The three landmarks are identified by small spheres on the surface (landmark 3 is hidden behind the edge of the plate).

Materials and Methods

Enallagma is one of the most speciose genera of odonates, with 38 Nearctic species and four Palearctic species (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, and provide a phylogenetic hypothesis on which the present study can be based (Brown et al. 2000; McPeek and Brown 2000; Turgeon et al. 2005). Six clades can be distinguished within the genus (Turgeon et al. 2005). Three sister clades each radiated within the last 250,000 years to produce approximately half of the extant species: the Nearctic "*hageni*" clade with 10 species, the Nearctic "*carunculatum*" clade with seven species, and the Palearctic clade with four. Two other clades have much longer evolutionary histories. The Orange clade (adults of these species are shades of red, orange, or yellow in color) contains eight species and has a last common ancestor dated to approximately 2 million years ago. The Blue/Black clade (adults of most of these species are colored blue and black) also contains eight species and has a last common ancestor dated to about 13 million years ago. A sixth Southwestern clade (most species are found in the southwestern US and Mexico) contains five species (we cannot date the last common ancestor of this clade because we do not have enough DNA sequence to accurately resolve relationships within this clade). For the analyses presented here, we use the molecular phylogenetic hypothesis presented in Turgeon et al. (2005, see also McPeek et al. 2008).

For this study, we obtained female specimens for 38 of the 43 species of *Enallagma*. The only missing species are the four Palearctic species and *E. truncatum* that is endemic to Cuba (Westfall and May 2006). All females were dried specimens (see Appendix for the localities where specimens were originally collected). Only one specimen was included per species, and we feel that this is justified by the following information: First, we examined the variability among five individuals of one species (*Enallagma aspersum*) relative to interspecific differences, and found that the variability among the *E. aspersum* individuals was small relative to interspecific differences. Specifically, the distances for *E. aspersum* individuals to their centroid were all smaller than the distances of the species to the centroid for all the species samples. Second, studies of the corresponding male cerci in four species showed no discernable interpopulation variation across their entire ranges, and intraspecific shape variation was also small relative to differences among species (McPeek et al. 2008; Shen et al., in press). Finally, the morphology of these structures are used by humans to unambiguously identify individuals to species, which also implies that intraspecific variability is insignificant relative to interspecific differences.

Each specimen was scanned using computer tomography (CT) technology in a SkyScan 1172 high-resolution micro-CT scanner (SkyScan, Kontich, Belgium). The thorax of each female was mounted on a brass stub with modeling clay and placed in the scanner. CT scans were made at a pixel resolution of 4.0 μm (i.e., voxel resolution of 64.0 μm^3) through 180°, with a rotation step of 0.7°/frame, and averaging three frames. CT scans were converted to stacks of digital image slices using NRecon version 1.4.4 software (SkyScan).

The left and right thoracic plates were segmented from the resulting digital image stack and initial processing was performed using Amira^{TM} version 4.1.2 software (Mercury Computer Systems Inc., Chelmsford, MA). All voxels associated with each plate were first identified using the editing and labeling tools. A high-resolution triangular mesh surface model of each plate was then constructed. For computational purposes, each triangular mesh surface model was reduced to have 10,000 triangles with 5002 vertices. The positions of three landmarks were also identified on each plate using Amira (Fig. 1). The female plates have a basic triangular outline with two of the points of the triangle being along the midline of the body, and the third point being lateral. The three landmarks identify the three points of this triangle. These landmarks were used to spatially register plates relative to one another.

We used spherical harmonics analyses of the triangular mesh surfaces to quantify female thoracic plate shape (Shen and Makedon 2006; Shen et al. 2009). We calculated the spherical harmonic representation of each surface using the algorithms described by Shen and Makedon (2006). Please also see McPeek et al. (2008) and Shen et al. (2009) for a fuller description of these algorithms and their applications to biological shapes. Spherical harmonics, an extension of the classic Fourier transform, represent a three-dimensional (3D) shape in terms of a sum of 3D sines and cosines on a sphere (Brechbühler et al. 1995; Ritchie and Kemp 1999; Shen et al. 2007). The result is a set of complex coefficients that quantify 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 (McPeek et al. 2008). The coefficients are estimated by solving a series of linear equations using standard least squares estimation. Including higher frequency components models greater detail of the structure. The *Enallagma* female plates were modeled to degree $l = 15$, which produces 256 ($= [l + 1]^2$) coefficients to represent each of the three spatial dimensions, for a total of 768 complex-valued coefficients. These algorithms were coded and run using Matlab version 7.4.0.287 (The Mathworks, Inc., Natick, MA); this code is available from the authors on request.

Prior to estimating the spherical harmonic coefficients, we standardized all plates to a common length measure to remove overall size from these representations. We chose to standardize all plates to a common length measure, because the contact between male cerci and female plates depends on distances between various points on the cerci and plates, whereas total surface area and volume have much less influence on the proper contacting of these structures (M. A. McPeek, unpubl. data). To do this, we rescaled the 3D positions of the vertices in each triangular mesh so that the distance between landmarks 1 and 3 (Fig. 1) was 1.0. Using other measures to standardize for size (e.g., square root of surface area) did not, however, alter the conclusions drawn from the analyses.

The resulting spherical harmonic coefficients provide a quantitative and orthogonal coordinate system to ordinate species based on shape. To visualize the positions of species in this phenotypic space, we performed a principal components (PC) analysis on the covariance matrix of the spherical harmonics coefficients using the princomp procedure of Matlab.

Evolutionary contrasts analyses were then used to quantify evolutionary rates of change in female plate shape by reconstructing the spherical harmonic coefficients along the phylogeny of the *Enallagma* (see Brown et al. 2000; Turgeon et al. 2005; and McPeek et al. 2008 for this phylogeny). Species for which we lacked plate models were pruned from the tree before analyses. 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). Because we are reconstructing the evolution of 768 coefficients for each structure in this analysis, we used multivariate evolutionary contrasts instead of the standard method. Multivariate evolutionary contrasts use the magnitude of the Euclidian distance between two taxa without regard to the direction of the difference as the numerator of the standardized contrast (Klingenberg and Ekau 1996; McPeek et al. 2008). 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 were calculated for each character, and then the distances between them were 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. 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 (see McPeek et al. 2008).

We tested whether the evolution of female plate shape was more consistent with a model of Brownian motion or a model of punctuated change by estimating κ in a maximum-likelihood framework (Pagel 1997; Freckleton et al. 2002). In this test, κ is a parameter used to rescale the branch lengths of the phylogeny by raising each branch length to the power of κ: the value of κ that best scales the branch lengths to fit the data is found using maximum-likelihood criteria (Pagel 1997). A value of $\kappa = 1.0$ leaves the branch lengths unchanged (i.e., all are raised to the power of 1), and implies that the model of character change that best fits the data is the Brownian motion model of continuous character change in which the phenotypic difference between two taxa scales directly with the amount of time they have evolved independently of one another. A best-fit value of $\kappa = 0.0$ would set all branch lengths of 1.0 (i.e., all are raised to the power of 0), which is consistent with a punctuated model of character evolution in which the amount of change associated with each branch is independent of its length. Because the traits studied here define species, we presumed that this punctuated change would have occurred at the time of speciation, if this model is most consistent with the data. We used the likelihood equation (eq. 4) of Freckleton et al. (2002) to search for the value κ that was most consistent with the multivariate distances between the female plate shapes of the *Enallagma* species using a standard search algorithm (Bessett 2001).

We also used the "censored" method of O'Meara et al. (2006) to estimate rates of character change and test for rate heterogeneity among the four major *Enallagma* clades for which we have >1 species (i.e., excluding the Southwest clade). For this analysis, we assumed the value of κ identified in the previous analysis. All evolutionary contrasts analyses were performed by a program written in Java 1.5 (Sun Microsystems) and are available from MAM upon request.

Results

We performed separate analyses for the left and right female plates, and because they are bilaterally symmetrical these two analyses were nearly quantitatively identical. For visualization of species relative to one another in the high-dimensional spherical harmonic space, the first two PCs explained ~46% of the total variation in female plate shape (the third PC explained only ~8% of the total variation) (Fig. 2A). The PC scores for each coefficient were complex numbers (spherical harmonic coefficients are complex numbers), but the imaginary parts for scores on the first several PCs were infinitesimal (on the order of 10^{-7}) relative to the real parts (on the order of 10°–10^{-2}). We therefore ignored the imaginary parts of the PC scores for this ordination (the imaginary parts of the PCs are not necessarily small relative to the real parts; this is only a fortuitous empirical result that we use here to look at these species in two dimensions). PC1 described a change in the relative length and width of the plates: plates with more positive scores on PC1 were broader in the anterior to posterior direction relative to the lateral length (Fig. 2B). PC2 described a change in the relative angle of the medial edge relative to the rest of the plate (Fig. 2B). The five clades did differ in their average positions

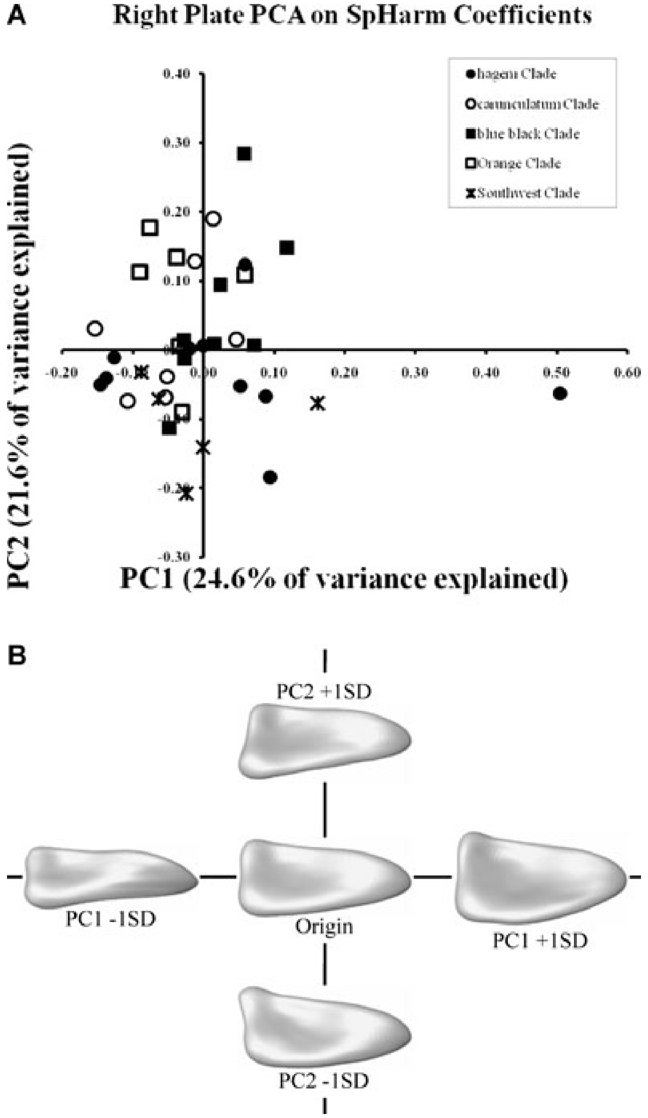

Figure 2. Principal components (PC) analysis of the spherical harmonic coefficients for the right female plates of the 38 *Enallagma* species. (A) The ordination of the species on the first two PC axes, which together explained 46.2% of the total variation in plate shape. Each symbol is the position of one species, and the different symbols identify the clade membership of species as given in the legend. (B) The variation in plate shape that is explained by each PC axis. The center object is the spherical harmonic model for the right female plate represented at the origin in the PC space. The anterior edge of the plate is up, the medial edge of the plate is to the left, and the view is of the dorsal surface. The objects to the left and right of the origin object are the spherical harmonic models for the right female plates 1 standard deviation in the negative and positive direction, respectively, along the PC1 axis (and at 0.0 on the PC2 axis). The objects above and below the origin object are the spherical harmonic models for the female plates 1 standard deviation in the negative and positive direction, respectively, along the PC2 axis (and at 0.0 on the PC1 axis). The PC analysis for the left female plates is nearly identical to the right plates.

in this reduced space (e.g., multivariate analysis of variance on the first two PCs for the right plates: Wilk's λ approximation, $F_{8,64} = 4.07$, $P < 0.001$) (Fig. 2A).

Although the clades differ in average position in the spherical harmonic morphospace today, the multivariate evolutionary contrasts analyses indicated that they arrived at their current diversities by similar tempos and modes of evolution. Our likelihood search found that a value of $\kappa \approx 0$ best fit the data (e.g., right plate $\kappa = 2.19 \times 10^{-10}$, $\log L = 28.72$), which is consistent with a model of punctuated character change (Pagel 1997). We also directly compared the punctuated model ($\kappa = 0.0$) to the Brownian motion model ($\kappa = 1.0$) and found that the punctuated model provided a much better fit to the data (likelihood-ratio test, $\chi^2_1 = 17.42$, $P < 0.001$). Assuming punctuated change (i.e., assuming all branches in the phylogeny have length 1.0), we also tested for evolutionary rate heterogeneity among the four clades using the multivariate shape contrasts (Table 1). Using traditional analyses of variance, the clades did not differ in the evolutionary rates quantified by the multivariate evolutionary contrasts (e.g., for the right plates, $F_{4,26} = 0.63$, $P > 0.60$). In addition, 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: $\chi^2_4 = 7.03$, $P > 0.10$; also, the model with a single rate for all clades had $\mathrm{AIC_c} = 22.84$, and the model with separate rates for each clade had $\mathrm{AIC_c} = 25.76$).

The multivariate evolutionary contrasts also demonstrated significant correlated evolution among these reproductive structures. Not surprisingly, the left and right female plates showed strong correlated evolution, because they are mirror images of one another ($r_{31} = 0.84$, $P < 0.001$). Multivariate evolutionary contrasts derived from the left and right male cerci in our previous

Table 1. Evolutionary rate estimates derived using the censored method of O'Meara et al. (2006) for the right female plate shape analyses. The Shape Multivariate column gives rate estimates derived for the multivariate contrasts of the spherical harmonic coefficients of shape. The first four rows of the table give the rate estimates assuming that each of the four major clades included in the analysis have separate rates. The final 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)

Clade	Species Richness	Shape Multivariate $\hat{\sigma}^2$
"*hageni*"	10	0.056
carunculatum"	7	0.035
Blue/Black	8	0.026
Orange	7	0.030
Entire Genus	37	0.028

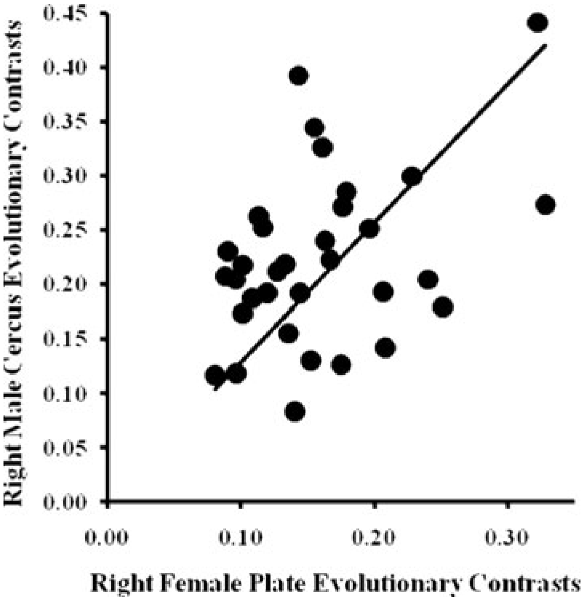

Figure 3. **Multivariate evolutionary contrasts analyses for the female plates and male cerci. The relationship between the multivariate evolutionary contrasts for the right male cerci and the right female plates is shown here. The relationship for the left structures is nearly identical, because the left and right male cerci and the left and right female thoracic plates are mirror images of one another, respectively.**

analysis (McPeek et al. 2008) also showed strong correlated evolution because they are also mirror images of one another ($r_{31} = 0.97$, $P < 0.001$). Most importantly, shape evolution in the male cerci and female plates were also significantly correlated (Fig. 3: $r_{31} \approx 0.38$, $P < 0.05$ for both left and right structures).

Discussion

Reproductive isolation in *Enallagma* is enforced primarily by the mating decisions of females. Adult females spend most of their time away from ponds and lakes foraging to support egg production, and only come to ponds to oviposit (Finke 1982). This behavior minimizes their exposure to harassing males and thus suggests an important role for sexual conflict in the evolution of mating behavior overall. Males spend almost all their time at ponds searching for females, and thus the operational sex ratio at ponds are highly male skewed (Fincke 1982, 1986). When she returns to oviposit, a female can be swarmed by multiple males of more than one species, who struggle to grasp her to initiate mating. Males do not discriminate among females of different species in their mating attempts (Paulson 1974; Miller and Fincke 2003; Fincke et al. 2007). Experiments have shown that a female accepts a male as a mate based on the tactile cues she receives from the male cerci as they contact the sensory setae of her thoracic plates (Paulson 1974; Robertson and Paterson 1982). Males cannot force females to mate, because the female must bend her abdomen up to be inseminated—females exercise ultimate choice of their mating partners (Fincke 1997).

Female mate choice based on the mechanical interactions between these morphological structures implies a kind of "lock-and-key" mechanism, although these are not genitalia (Dufour 1844; Shapiro and Porter 1989): the tactile cues a female receives when grasped by a male will depend on the distribution of contact areas and forces defined by the mechanical interaction of her thoracic plates with the male's cerci. Thus, correlated evolution in these structures is expected if the interaction of these parts is important in defining the boundaries of premating reproductive isolation. Previously, we showed that change in *Enallagma* male cerci shape was consistent with an evolutionary model of punctuated change at the time of speciation and homogenous rates of change across the clade (McPeek et al. 2008). The results of the present study also demonstrate that change in the shape of female plates was consistent with the model of punctuated change at the time of speciation and homogeneous rates of change across the clade. Moreover, the multivariate evolutionary contrasts of shape change were correlated with one another, confirming that male cerci and female thoracic plates have evolved in a correlated fashion across the genus.

These evolutionary dynamics imply that these structures change rapidly at the time of speciation, but change little in the intervening periods between speciation events (Templeton 1979; Paterson 1993). Given that these structures define species boundaries, rapid change at the time of speciation is not surprising. After differentiation is accomplished, female mate choice should impose strong stabilizing selection on male cerci shape, because more extreme cerci phenotypes will be less likely to mate (Lande 1981, 1982; Sved 1981a,b). Consistent with this, we have found no interpopulation variation in cerci shape across the ranges of four *Enallagma* species so far examined (McPeek et al. 2008; Shen et al., in press). Likewise, the distribution of male phenotypes in a population may in turn impose some degree of stabilizing selection on female plate shape, because females with more extreme plate shapes will also be less likely to find suitable mates and males may have greater difficulty maintaining hold of such females and be more willing to terminate mating before insemination.

If the process of mate recognition imposes strong stabilizing selection on both of these structures, what is the impetus for the rapid evolutionary change that would result in speciation? In some species, such rapid differentiation of premating reproductive isolating mechanisms is thought to evolve to reduce the costs of producing less-viable or less-fertile hybrids when mating with heterospecifics that are already partially differentiated (Kelly and Noor 1996; Kirkpatrick and Servedio 1999; Kirkpatrick 2000, 2001). This is probably not so for many interacting *Enallagma*.

Each of the three most recently radiated clades shows a very high degree of unsorted polymorphism at mitochondrial loci among most species within each radiation, with some common haplotypes being found in up to five species (Turgeon et al. 2005). In addition, although a few of the speciation events in these clades were the result of ecological differentiation, most involved little, if any, ecological differentiation (McPeek and Brown 2000; Stoks and McPeek 2006). Moreover, many of these species can be found living together at ponds and lakes across large areas of North America. However, almost all are differentiated at more rapidly evolving AFLP loci, and so are good "biological" species (Turgeon et al. 2005). Because mitochondrial genes are some of the fastest evolving genes in animals and most species within each radiation can only be distinguished based on their cerci and thoracic plates, species within each radiation may be quite genetically compatible with one another. Thus, differentiation of male cerci and thoracic plates would not seem to have been driven by "reinforcement" during secondary contact (e.g., Kelly and Noor 1996; Kirkpatrick and Servedio 1999; Kirkpatrick 2000, 2001).

A number of other processes have been proposed to be capable of generating such rapid changes in female choice: among them are founder events (Templeton 1979; Carson and Templeton 1984; Gavrilets and Boake 1998), the interaction of natural and sexual selection (Lande 1981, 1982; Kirkpatrick and Ravigné 2002) and species interactions (Payne and Krakauer 1997; Gavrilets 2004; McPeek and Gavrilets 2006). Most of these involve populations experiencing some sort of novel conditions: for example, founder events occur when a species colonizes a new location. Biogeographic and phylogenetic data suggest that *Enallagma* may have repeatedly experienced such novel conditions because of punctuated climate change associated with glacial advances and retreats throughout the Pleistocene. For example, most species in the two North American radiations have species' ranges that would have been almost completely under ice at the last glacial maximum (Donnelly 2004). Moreover, all these species show genetic signatures of range fragmentations and expansions, and many can be dated to glacial advances or retreats (Turgeon et al. 2005). Range fragmentation and rapid range expansions could both change the regime of natural selection experienced by species that would interact with sexual selection (Lande 1981; Gavrilets 2004) and generate genetic drift in female plate shape in local populations (Templeton 1979; Carson and Templeton 1984; Gavrilets and Boake 1998; Regan et al. 2003) to create new species.

In addition, we have identified two areas of North America where unidirectional mitochondrial hybridization occurred briefly in the recent past between members of the two radiating clades (Turgeon et al. 2005). Unidirectional mitochondrial gene flow is characteristically seen in situations in which females of one species are locally rare and cannot find conspecific males for mates; they eventually acquiesce to the mating advances of locally common heterospecific males (Wirtz 1999; Randler 2002). These types of social situations must have been quite common as *Enallagma* species colonized the lakes formed in newly deglaciated areas after each glacial retreat during the Pleistocene. Given that local population size can be on the order of 10^5–10^7 individuals at any given pond or lake, females of later colonizing species would initially be interacting primarily with the heterospecific males of previous colonists. This social situation can impose strong directional selection on female preferences in the rare colonist species to differentiate rapidly from the earlier-arriving species (McPeek and Gavrilets 2006: and see also Hoskin et al. 2005; Pfennig and Pfennig 2005 for empirical examples). This scenario could have played out multiple times across the entire continent during every glacial retreat in the Pleistocene to create multiple new species simultaneously. Recolonizing deglaciated areas may have thus imposed substantial selection for differentiation in female plate morphology directly through this mechanism.

Many different types of traits are used by males and females both to discern the species identity of potential mates and to discriminate among potential conspecific mates: e.g., morphology (Eberhard 1985; Shapiro and Porter 1989), breeding coloration (e.g., Seehausen et al. 1997; Ready et al. 2006), mating songs (e.g., Ryans and Rand 1993; Henry et al. 1999; Shaw 2000; Gerhardt 2005), sexual pheromones (e.g., Blows 2002), behavioral repertoires (e.g., Boake 2002). Although these types of traits have received intensive functional and genetic scrutiny at the intraspecific level, we know very little about the macroevolutionary dynamics of such traits as clades have diversified, and how these patterns of diversification match with current patterns of intraspecific variation. Arnqvist and Rowe (2002a,b) in their analyses of sexual conflict armaments and defensive structures in the clade of *Gerris* waterstriders have shown that many features of overall body shape as well as specific sexual structures, including female defensive spines and male genital length, change in a correlated evolutionary fashion between the sexes; they assumed a model of punctuated change at the time of speciation in their analysis. Likewise, Brennan et al. (2007) showed a phylogenetic correlation between vagina length and phallus length in waterfowl, assuming Brownian motion evolution of traits (i.e., branch lengths proportional to molecular genetic distances among species). It would be interesting to extend these studies to estimate the best-fit model of character change (Pagel 1997). Because sexual selection and sexual conflict are expected to generate more continuous change both among populations and through time (Andersson 1994; Arnqvist and Rowe 2005; Hosken and Stockley 2003), we predict that traits evolving primarily due to intraspecific sexual selection or sexual conflict should show macroevolutionary dynamics of continuous character change (i.e., $\kappa = 1$ [Pagel 1997]) and variability among populations within species. In contrast, traits primarily involved in discriminating conspecifics from heterospecifics should evolve according to a punctuated model of evolutionary change (i.e., $\kappa = 0$) and show little population differentiation across the entire range of a species, as in *Enallagma* (McPeek et al. 2008; unpubl. ms.).

Sexual traits need not function exclusively as signals of either species identity or intraspecific sexual attractiveness, but different traits may primarily influence one or the other (e.g., Doherty 1985; Ryan and Rand 1993, 2001; Boake et al. 1997; Hankison and Morris 2003; Castellano and Cermelli 2006; Phelps et al. 2006). Because the morphologies of genitalia and other sexual structures like cerci and female thoracic plates are used by taxonomists to define species in many arthropod groups, many have taken this as circumstantial evidence that these structures are used as species recognition traits (Eberhard 1985; Huber 2003). However, studies of many different groups have produced inconsistent results. For example, some taxa have polymorphic genitalia (e.g., Hausmann 1999; Huber and González 2001; Jocqué 2002; Mutanen and Kaitala 2006), genital morphology varies much more than expected in some taxa (e.g., Sirot 2003; Polihronakas 2006; Song and Wenzel 2008), and specific tests of hypotheses concerning the modes of species specificity (e.g., the lock-and-key hypothesis) are often not supported (e.g., Eberhard 2005; Mutanen et al. 2006). Moreover, the signatures of sexual selection operating on genital morphology can be detected in some taxa (e.g., Arnqvist 1997; Fairbairn et al. 2003; Bertin and Fairbairn 2005; reviewed in Hosken and Stockley 2003). A particular mechanism cannot be assumed to operate on any particular type of trait in all taxa. Rather, competing hypotheses must be rigorously tested, and comparative analyses can aid in this discrimination. In doing so, we will develop a deeper understanding of how these processes shape patterns of biodiversity.

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Appendix. **Locality and collection date for the *Enallagma* specimens included in the Interspecific analysis of cerci shape.**

Genus	Species	Location	Date Collected
Enallagma	*anna*	Bloody Creek and County Line Road, Loup Co., Nebraska	20 July 2005
Enallagma	*annexum*	Sevy Bog 1.5 miles N of Sevy Corners, St. Lawrence Co., New York	9 June 2003
Enallagma	*antennatum*	Chemung River, Bottcher's Landing, Big Flats, Chemung Co., New York	3 July 2004
Enallagma	*aspersum*	Pond "8," Enfield Corner's Road, Enfield, Tompkins Co., New York	1 July 2003
Enallagma	*basidens*	San Sabo River, 0.7 miles NE of Fort McKavitte, Menard Co., Texas	15 July 2001
Enallagma	*boreale*	Colton, 3 miles SW of Orebed Road, St. Lawrence Co., New York	5 June 2005
Enallagma	*cardenium*	West Branch Hog Town Creek at NW 16th Ave. Gainesville, Alachua Co., Florida	18 July 1969
Enallagma	*carunculatum*	Lebanon Road and Chenango River, Earlsville, Madison Co., New York	18 July 2005
Enallagma	*civile*	13 miles N of Gothenberg, Rt 47, Custer Co., Nebraska	17 July 2005
Enallagma	*clausum*	Rye Patch Reservoir, North of I-80, Pershing Co., Nevada	26 July 1978
Enallagma	*coecum*	Pond NE of Buena Vista and north of Hotel Montana, La Vega Province, Dominican Republic	24 May 1991
Enallagma	*concisum*	Marsh 1.5 miles N of entrance to Blackwater River State Park, Santa Rosa Co., Florida	12 April 2004
Enallagma	*daeckii*	Blackwater River State Park, Santa Rosa Co., Florida	13 April 2004
Enallagma	*davisi*	Lake Galilee, Gainesville, Florida	17 March 1971
Enallagma	*divagans*	Beaver Pond, Bear Brook State Park, Suncook, New Hampshire	29 June 2004
Enallagma	*doubledayi*	Rt. 66 and road to Lake Charlotte, Highlands Co., Florida	24 March 2004
Enallagma	*dubium*	West branch of Hatchet Creek, Highway S-225 Alachua Co., Florida	24 August 1982
Enallagma	*durum*	Hudson River C Alpine Boat Basin, Pallisades Interstate Park on Rt. 9W north, Alpine Township, Bergen Co., New Jersey	18 August 1991
Enallagma	*ebrium*	Colton, 3 miles SW of Orebed Road, St. Lawrence Co., New York	5 June 2005
Enallagma	*eiseni*	Sur, pond at Rancho San Enrique 51 km E of Villa Insurgentes, Baja California, Mexico	4 October 1984
Enallagma	*exsulans*	Bloody Creek and County Line Road, Loup Co., Nebraska	20 July 2005
Enallagma	*geminatum*	Stone Mill Pond, Madison Co., New York	14 July 1999
Enallagma	*hageni*	2.5 miles SE of Redcoach Quarry, Naugatuck, New Haven Co., Connecticut	22 June 1997
Enallagma	*laterale*	Lovell's Pond, Santoit/Newtown Road, Marstons Mills, Massachusetts	10 June 1995
Enallagma	*minusculum*	White Lake, Bladen Co., North Carolina	30 May 1966
Enallagma	*novaehispaniae*	Domatila new Rio Dorado, Granada Department, Nicaragua	27 August 2003
Enallagma	*pallidum*	Gordonia-Alatamaha State Park, US Highway 280, Reidsville, Tattnal Co., Georgia	14 May 2005
Enallagma	*pictum*	Whitesbog, Lebanon State Forest, Burlington Co., New Jersey	8 June 1991
Enallagma	*pollutum*	Francis Taylor WMA at Rt. 41, 12 miles west of Rt. 997, Dade Co. Florida	3 April 2005
Enallagma	*praevarum*	Oak Creek, Page Spring, Yavapai Co. Arizona	3 September 1983
Enallagma	*recurvatum*	Cedar Lake, SR 347 and 550 Spur, Bellplain State Forest, Cape May Co., New Jersey	22 May 1995
Enallagma	*semicirculare*	9.9 miles east of Buasave turnoff on Mex. 15, Sinola, Mexico	1 September 1965
Enallagma	*signatum*	Roger Nature Center, Sherbourne, Chenango Co., New York	13 July 1999
Enallagma	*sulcatum*	Lake June, Lake Placid, Highlands Co., Florida	11 June 1961
Enallagma	*traviatum*	Pond "18," Co. Rd. 6, Hector, Schyler Co., New York	1 July 2002
Enallagma	*vernale*	McDaniels Marsh, Enfield, New Hampshire	11 June 2007
Enallagma	*vesperum*	McDaniels Marsh, Enfield, New Hampshire	19 July 2005
Enallagma	*weewa*	Peaslee Wildlife Management Area, Tuckahoe River, Upper Township, Cape May Co., New Jersey	23 July 1990