Genetic Differentiation, Structure, and a Transition Zone among Populations of the Pitcher Plant Moth *Exyra semicrocea*: Implications for Conservation

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Abstract

Pitcher plant bogs, or carnivorous plant wetlands, have experienced extensive habitat loss and fragmentation throughout the southeastern United States Coastal Plain, resulting in an estimated reduction to <3% of their former range. This situation has lead to increased management attention of these habitats and their carnivorous plant species. However, conservation priorities focus primarily on the plants since little information currently exists on other community members, such as their endemic arthropod biota. Here, we investigated the population structure of one of these, the obligate pitcher plant moth *Exyra semicrocea* (Lepidoptera: Noctuidae), using mitochondrial cytochrome c oxidase subunit I (COI) gene sequences. Examination of 221 individuals from 11 populations across eight southeastern US states identified 51 unique haplotypes. These haplotypes belonged to one of two divergent (~1.9–3.0%) lineages separated by the Mississippi alluvial plain. Populations of the West Gulf Coastal Plain exhibited significant genetic structure, contrasting with similarly distanced populations east of the Mississippi alluvial plain. In the eastern portion of the Coastal Plain, an apparent transition zone exists between two regionally distinct population groups, with a well-established genetic discontinuity for other organisms coinciding with this zone. The structure of *E. semicrocea* appears to have been influenced by patchy pitcher plant bog habitats in the West Gulf Coastal Plain as well as impacts of Pleistocene interglacials on the Apalachicola-Chattahoochee-Flint River Basin. These findings, along with potential extirpation of *E. semicrocea* at four visited, but isolated, sites highlight the need to consider other endemic or associated community members when managing and restoring pitcher plant bog habitats.

Introduction

Currently, global biodiversity losses are occurring at an alarming rate, exceeding anything in the geological past [1]. These losses have further implications as they can exacerbate collapses of ecosystem functions. The most recognized and potentially well-documented examples of biodiversity and ecosystem loss have been in the tropics, home to half of the world’s species [1]. However, less well known is the fact that temperate zone ecosystems including marine [2], freshwater [3], and terrestrial [4] environments are being adversely impacted at an ever faster rate. Given that the majority of the world’s affluent human populations have historically resided in the temperate region [5,6], it is not surprising that many of its ecosystems have experienced anthropogenic driven environmental degradation, extensive species declines and habitat loss [7–9]. Such negative anthropogenic influences are well documented to have occurred in the United States, which has ~38 threatened, 58 endangered, and more than 30 critically endangered ecosystems [10].

Among the most critically endangered in the United States is the longleaf pine ecosystem, which historically occupied most of the southeastern Coastal Plain [10]. Estimates suggest that there has been a >98% decline of pre-European settlement longleaf pine (*Pinus palustris*) forests in this area [11]. Encompassed within the longleaf pine ecosystem are a myriad of endangered habitats including carnivorous plant wetlands, commonly referred to as pitcher plant bogs. These bogs are typically found in wet pine flatwoods or seepage slopes and are characterized by wet, sandy and low nutrient soils [12]. In these habitats, many endemic plants have evolved a carnivorous lifestyle, obtaining nutrients through the capture and digestion of animal prey. Similar to the overall ecosystem, current estimates suggest that the pitcher plant habitat now occupies <3% of its former range largely due to restriction of natural fires, urbanization, forestation and agriculture [12]. Of the more than 29 carnivorous plant species representing five genera [13], at least five species are federally endangered or threatened [14] and all species in the genus *Sarracenia* (pitcher plants) are listed...
in The Convention on International Trade in Endangered Species (CITES, www.cites.org).

Many studies on carnivorous plants have focused on the investment in and evolution of carnivory [15–17] that was pioneered in part by Charles Darwin’s fascination with the subject [10]. Other areas of interest include the genetics and population structure of these plant species since such information has implications for conservation of these unique organisms. For example, varying levels of genetic diversity have been reported across Sarracenia sp. and populations [19–21]. Additionally, recent research examining Sarracenia alata (the winged pitcher plant), which ranges from eastern Texas to western Alabama, identified a phylogeographic break and genetic divergence centered on the Mississippi River alluvial plain, with additional population structure on either side of the break [22]. Such results highlight the potential uniqueness of individual pitcher plant populations that should be considered when developing management plans for these habitats.

While research and conservation efforts have primarily focused on the carnivorous plants, less attention has been given to other arthropods that inhabit Sarracenia. For example, a number of arthropods have evolved a mutualistic relationship with these bogs support a complex and intimately associated biotic community. For example, a number of arthropods have evolved characteristics for inhabiting Sarracenia sp. [12], including >17 species of endemic mites, flies and moths [15,25,24]. Of these, the herbaceous moth Erya semicrocea (Lepidoptera: Noctuidae) ranges across the United States southeastern Coastal Plain and from eastern Texas to southern Virginia [25,26] and is of conservation concern due to its obligate relationship with Sarracenia sp. like S. alata [25,27]. Specifically, E. semicrocea is oligophagous, feeding exclusively on Sarracenia sp., and its life cycle occurs entirely inside pitcher plant leaves, from oviposition through larval development and mating. Because of this well documented relationship, we believe that the E. semicrocea/Sarracenia complex represents an ideal system for examining ecological and biogeographical questions pertaining to pitcher plant bogs and their endemic biota. Here, we examined populations of E. semicrocea across the southeastern Coastal Plain to address the hypothesis that the genetic structure of this moth is consistent with the major finding of Koopman and Carstens [22] for S. alata, namely that a strong genetic break occurs across the geographic region occupied by the Mississippi River alluvial plain.

Methods

Ethics Statement

This study was conducted in accordance with all United States laws and internationally accepted protocols with relevant collecting permits from The Nature Conservancy (for sites Centre Bog, Eller Seep, Green Swamp, Reed Branch), National Estuarine Research Reserve Sites (covered under Award/Permit #NA1100S#2000538; for sites Apalachicola, Grand Bay, Weeks Bay), Big Thicket National Preserve Site (Permit # BITH-2010-SCI-0006; for site Big Thicket), and Okfenokee National Wildlife Refuge (Permit # 41590-10-033; for site Okfenokee).

Sample techniques, DNA extraction and polymerase chain reaction (PCR)

Specimens of E. semicrocea were collected across eight southeastern Coastal Plain states between May and August 2010 (Figure 1). Erya semicrocea was not found at four of the visited sites (Centre, AL; Prattville, AL; Reed Branch, GA and Eller Seep, NC); thus, samples were acquired from 11 localities. At each site, attempts were made to collect a minimum of 24 E. semicrocea individuals, however, the actual number acquired per population ranged from 3–24 (x = 21) specimens. Individuals were preserved in 95% ethanol in the field and DNA extraction from each E. semicrocea was performed with the method described by Collroth et al. [20]. The resulting DNA extractions served as templates for amplifying a ~600 bp fragment of the mitochondrial (mtDNA) cytochrome c oxidase subunit I (COI) gene. The primer pair for subsequent polymerase chain reaction (PCR) was LCO1490 (5'-GGTCAA-CAXTCAATAAAGATATTGG-3') and HCO2198 (5'-TAAACC-TTCGGAGGTGACCXXXATCA-3') [29]. The COI region was chosen because it can reveal both historical and current genetic patterns [30] and has been shown to be informative in other Lepidoptera species [31,32]. Each PCR was conducted in 25 µl volumes containing ~10–30 ng of template DNA, 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 0.001% gelatin, 2.0 mM MgCl2, 200 mM dNTPs, 1 U Taq DNA polymerase and 0.4 mM of each primer. PCR was conducted with a PTC-100 Thermal Cycler (MJ Research) using the following cycling profile: initial denaturing step of 94°C for 5 min, 15 cycles of 94°C for 45 s, 40°C for 45 s, 72°C for 60 s; 25 cycles of 94°C for 45 s, 55°C for 45 s, 72°C for 60 s, and a final extension of 72°C for 5 min. Five µl aliquots of PCR product from each reaction were electrophoresed in 1% agarose gels, stained using ethidium bromide, and viewed using shortwave (265 nm) UV to confirm PCR success. Prior to sequencing, amplification products were purified with Montage™ PCR Filter Units (Millipore) according to the manufacturer’s specifications. Sequencing was conducted in both directions using Big-Dye Terminators v.3.1 and analyzed on a PRISM 3100 (Applied Biosystems, USA) at the Auburn University Genomics and Sequencing Laboratory or the University of Washington Genomics Unit. Forward and reverse sequence chromatograms were assembled and any ambiguities corrected using Sequencer v.4.8 (Gene Codes Corporation, USA). All finished sequences were aligned manually using SE-AL version v2.0a11 (available at http://tree.bio.ed.ac.uk/software/seal/).

Data analysis

Genetic diversity, population structure and migration. Estimates of population genetic diversity were obtained using haplotype (h) and nucleotide (π) diversities calculated by Nei’s [33] method in the program DnaSP v5.10.01 [34]. Population structure was estimated using two approaches. First, the Snn or ‘nearest-neighbour’ statistic [35,36] was calculated as Nei’sgst/2 for all populations, thus the results presented here focus specifically on genetic structure between geographic sites. To quantify the spatial distribution of genetic variation in E. semicrocea, analyses of molecular variance (AMOVAs) [39] were conducted with Arlequin. Estimates of the relative contribution of molecular variance were assessed at three hierarchical levels using Φ statistics: (i) between groups (ΦST), designated by the resulting population structure from pairwise ΦST and Snn statistics; (ii) among populations within groups (ΦSC); and (iii) within populations (ΦCT). The significance of both the pairwise ΦST statistics and AMOVAs were assessed with 10,000 permutations. All of the above tests, when applicable, were conducted under
Tamura and Nei’s [40] model of nucleotide evolution with rate variation among sites [TN+I] as selected by the Akaike Information Criterion (AIC) in ModelTest v3.6 [41]. Lastly, Mantel [42] and 3-way (partial) Mantel [43] tests were conducted with the Vegan package [44] in the R v2.6.2 statistical software environment [45] with 10,000 permutations. A standard Mantel test was utilized to assess whether genetic and geographical distances are correlated. To determine if a potential transition zone among eastern populations influences the relationship between genetic and geographical distances, partial Mantel tests were conducted with an additional binary matrix defining populations as belonging to one of two groups (see Results).

To discriminate between the relative effects of ongoing migration (M = 2N_em) versus recent divergence between pairs of E. semicrocea populations, a Markov Chain Monte Carlo (MCMC) based analytical method was used as implemented in MDIV [46] (available at http://cbsuapps.tc.cornell.edu/). Analyses were conducted under the finite-site mutation model, which incorporates the possibility of multiple mutations per site, differences in nucleotide frequencies and the presence of transition/transversion bias. Three independent runs were conducted with the following conditions: M_max = 50, T_max = 10, length of Markov chain = 2 × 10^6 cycles, burn-in time = 5 × 10^5 cycles. Different random seeds were used in each run to check for consistency in the estimates.

**Haplotype network and demographic analyses.** Networks were generated to depict relationships among E. semicrocea haplotypes, based on the cladogram estimation algorithm of Templeton et al. [47], with TCS v1.21 [48]. The networks were constructed under the default setting (i.e., 95% parsimonious plausible branch connections). Reticulations in the networks, implying ambiguous connections between haplotypes, were resolved according to the suggestions of Crandall et al. [49]. Two approaches employing Fu’s Fs [50] and Tajima’s D [51] were used to determine if patterns of genetic variation observed in the COI sequences were consistent with predictions under a neutrality model. While these tests are typically employed to detect selection [50,51], they can also prove informative regarding the demographic history of a population [52]. Both neutrality tests were conducted in Arlequin and significance (P<0.05) assessed by 10,000 permutations.

**Results**

**Genetic diversity of Exyra semicrocea**

A total of 221 Exyra semicrocea were sampled from 11 localities across a range of ~1,700 km. Overall, 51 (8.1%) nucleotide
positions across the analyzed 630 bp COI fragment were variable and no stop codons were encountered. Nucleotide differences at the majority of these sites (50) represented ‘silent’ substitutions (i.e., would not change the encoded amino acid), with the remaining one being a non-synonymous substitution to an amino acid with similar biochemical properties (data not shown). These patterns suggest that all sequences were derived from mitochondrial copies of COI rather than nuclear copies of mitochondrial derived genes (numts) [53,54]. From the 221 total individuals sampled, 54 unique haplotypes were identified with 34 occurring as singletons and the remaining 23 being identified more than once (See Table S1). Sequences were deposited in GenBank under accession numbers HQ646110–HQ646163.

Haplotype frequencies within populations varied from 2–15, with haplotype (h) and nucleotide (π) diversities ranging between 0.51–0.94 and 0.00097–0.00434, respectively (Table 1). Genetic distances between any two haplotypes ranged from 1 (0.2%) to 19 (3.0%) variable sites, with sequence divergence across the Mississippi River alluvial plain being between 1.9–3.0% (x = 2.5%). Arthropod COI mutation rates have been estimated at 2.3% sequence divergence per million years [55], and more specifically, between 0.78–1.02% per million years for the family Papilionidae (Lepidoptera) [56]. Use of these rates yield an estimate that populations separated by the Mississippi River alluvial plain diverged from each other between 1.0–3.2 mya. Furthermore, none of the identified haplotypes were shared between populations across the Mississippi River alluvial plain (Figure 2). Thus, all subsequent population genetic analyses treated the western and eastern populations of E. semicrocea separately.

**Genetic differentiation, structure, and migration rates**

Pairwise ΦST and Snn statistics revealed significant genetic differentiation in 46 of 62 pairwise population comparisons of E. semicrocea (Tables 2 and 3). For example, all three populations west of the Mississippi River alluvial plain (KN, BT and AN; Fig. 1) were significantly differentiated from one another for both estimates, with comparisons between BT and KN (~150 km) and BT and AN (~73 km) having Snn values of 1.0 (Table 2). This implies a complete lack of haplotype exchange between these populations. Estimates of migration (M) via mtDNA further support this, suggesting no female (since mtDNA is maternally inherited) migrants (M = 0) had been exchanged between the BT and KN populations in the recent past (data not shown). An analysis of molecular variance (AMOVA) found a significant proportion of genetic variation occurring between populations (i.e., BT vs. AN/KN; ~55%; ΦCT = 0.552; P < 0.05). Additionally, estimates for among populations within groups and within populations were significant (P < 0.01) and accounted for ~24% and ~21% of the total observed genetic variation, respectively (Table 4).

Comparisons among the eastern populations found a lack of genetic structure among AC, GB, and WB for both the pairwise ΦST and Snn statistics (Table 3). For AP, OF, FM, and GS, weak but significant structure was detected depending on the statistical estimate (Table 3). In contrast, both statistics identified CF as being distinct from nearly all other sites with the exception of AC (Table 3). Therefore, subsequent genetic analyses were conducted on the eastern population groups of AC/GB/WB, AP/OF/FM/ GS and CF. In this context, a significant proportion of variation was partitioned within populations (~52%; ΦST = 0.523; P < 0.001) and among groups (~47%; ΦCT = 0.469; P < 0.05), with a small (i.e., 0.75%; ΦST = 0.014) but significant (P < 0.001) component among populations within groups (Table 4). Furthermore, a Mantel test identified an overall positive (r = 0.542) and significant (P = 0.013) correlation between genetic and geographical distances. Because of the distinct geographic clustering observed among specific eastern populations, a 3-way Mantel test was conducted in order to evaluate the relative contributions of contemporary isolation-by-distance versus historical isolation to the overall pattern. For this analysis, a third binary (i.e., 1 = both populations

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**Table 1. Genetic diversity and neutrality tests for Exyra semicrocea populations across the southeastern United States Coastal Plain.**

| Geographic region | Population | Genetic diversity | Neutrality tests |
|-------------------|------------|-------------------|-----------------|
|                   | n | nh | π   | h   | Tajima’s D | Fu’s Fs |
| West Mississippi R. |  |    |     |     |          |        |
| AN                | 3 | 2  | 0.00317 | 0.66667 | 0.000 | 1.609 |
| BT                | 24| 5  | 0.00189 | 0.67754 | 0.309 | −0.432 |
| KN                | 24| 6  | 0.00149 | 0.70290 | −0.867 | −2.162* |
| Total             | 51| 13 | 0.00520 | 0.86510 | 0.158 | −1.921 |
| East Mississippi R. | |  |     |     |          |        |
| AC                | 24| 14 | 0.00411 | 0.89855 | −1.548* | −7.981* |
| GB                | 23| 14 | 0.00305 | 0.91700 | −2.002* | −10.786* |
| WB                | 24| 7  | 0.00167 | 0.55797 | −1.957* | −3.265* |
| CF                | 24| 8  | 0.00434 | 0.89130 | 0.437 | −7.981* |
| AP                | 24| 15 | 0.00400 | 0.91304 | −1.465 | −9.957* |
| OF                | 21| 5  | 0.00230 | 0.64286 | 0.125 | −0.102 |
| FM                | 15| 4  | 0.00160 | 0.71429 | 0.280 | −0.064 |
| GS                | 15| 3  | 0.00097 | 0.51429 | −0.024 | −0.064 |
| Total             | 170| 44 | 0.00457 | 0.89795 | −1.632* | −26.374* |

| ns, number of sampled individuals. |
| nh, number of recovered haplotypes. |
| p, nucleotide diversity. |
| h, haplotype diversity. |
| *P < 0.05. |

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in same group, 0 = different group) matrix was created and the 3- way Mantel test detected a significant correlation ($r = 0.899, \ P = 0.024$) between genetic distance and this binary (i.e., the matrix defining the two groups with 3–4 populations, CF was excluded) but no significance correlation ($r = 0.122, \ P = 0.218$) between the geographic and genetic distance matrices. These results suggest that historical isolation, rather than geographic

Figure 2. Map and corresponding haplotype networks for the western and eastern lineages of *Exyra semicrocea* across the southeastern United States Coastal Plain. Each circle in a network represents a unique mitochondrial DNA (mtDNA) cytochrome c oxidase subunit I (COI) haplotype, with circle size corresponding to haplotype frequency. Rectangles represent the inferred ancestral haplotype or the haplotype with the highest outgroup probability according to the rcs analyses. Each branch, in spite of length, represents one mutational difference between haplotypes, with black circles indicating unsampled (i.e., missing) haplotypes. Colors represent localities designated by matching stars, which correspond to sampling sites specified in Figure 1.

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Table 2. Measures of genetic differentiation for western populations of *Exyra semicrocea* in Texas and western Louisiana as estimated by $S_{st}$ (upper right triangle) and pairwise $\Phi_{ST}$ (lower triangle) statistics.

| Populations | KN | BT | AN |
|-------------|----|----|----|
| KN          | –  | 1.000*** | 0.920* |
| BT          | 0.800* | – | 1.000*** |
| AN          | 0.548* | 0.694* | – |

* $P < 0.05$; *** $P < 0.001$.

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Due to the occurrence of CF within the proposed transition zone (Figure 2), pairwise estimates of migration ($M$) between all eastern populations were conducted relative to both AP and WB (i.e., the closest populations to CF). Population comparisons on either side of the potential transition zone (i.e., within groups) revealed plateaus in the distribution of posterior probabilities of $M$ (Figure 3), suggestive of high levels of intra-regional migration and consistent with the lack of genetic structure implied by the $S_{st}$ and $\Phi_{ST}$ statistics for these populations (see above). Conversely, clear peaks in the distribution of posterior probabilities for $M$ were identified in population comparisons across the proposed transition zone (i.e., between groups; Figure 3). For example, population comparisons of WB relative to GS, FM, OF, and AP (i.e., across groups) produced estimates for $M$ of 0.2, 0.2, 0.3, 0.5, respectively, with plateaus for AC and GB (i.e., within groups; Figure 3A). These results were inverted for population comparisons of AP (Figure 3B), which is located on the opposite side of the proposed transition zone (Figure 2). In both cases, CF remained an outlier
with an estimated $M$ approximately double that of population comparisons across the proposed transition zone (i.e., between groups; Figure 3). Again, these estimates of $M$ imply a distinct shift in the level of migration in relation to the proposed transition zone, a pattern consistent with the outcome of the 3-way Mantel test.

Demography of *Exyra semicrocea*

Populations west of the Mississippi River alluvial plain had both positive and negative values for Tajima’s $D$ and Fu’s $F_s$, with KN being the only population with a significant negative $F_s$ value (Table 1). In contrast, five of eight populations east of the Mississippi River possessed significant negative $F_s$ values, with three (i.e., AC, GB, WB) also significant for Tajima’s $D$ (Table 1). Significant negative values indicate an excess of rare polymorphisms within each of these populations and implied either positive selection or recent population expansion [51,52]. Due to the near total absence of non-synonymous substitutions in the analyzed COI fragment (see above), it is surmised that recent population expansion is the most likely explanation for these values. Taken together, populations of *E. semicrocea* in the eastern portion of its range as a relative whole are undergoing expansion compared to those west of the Mississippi River alluvial plain (Table 1).

**Discussion**

West Gulf Coastal Plain and Mississippi River alluvial plain

Populations of *Exyra semicrocea* in the West Gulf Coastal Plain (WGCP, USGS designation) possess appreciable divergence from, and a clear break between, eastern populations across the Mississippi River alluvial plain. The identification of the Mississippi River alluvial plain as a significant barrier has been documented for a number of species [57,58], including other members of the longleaf ecosystem, such as *Pinus palustris* (longleaf pine), *Dichromona colorata* (white topped sedge), *Drosera capillaris* (pink sundew), *Rhexia alifanus* (a meadow beauty), and *Utricularia* sp. (bladderworts) [59]. Notably, an identical break is present in the host plant of western *E. semicrocea* in this region (i.e., *Sarracenia alata*) [22] and results from an, 200 km wide swath of unsuitable soils and alluvial swamps replacing bog habitat in this region [60]. Given that *S. alata* minimally overlaps with other *Sarracenia* sp. throughout its range [61] and is the only host plant for populations of *E. semicrocea* in Texas and Louisiana, it is not surprising that this

### Table 3. Measures of genetic differentiation for eastern populations of *Exyra semicrocea* as estimated by $S\text{nn}$ (upper right triangle) and pairwise $\Phi_{ST}$ (lower triangle) statistics.

| Populations | AC | GB | WB | CF | AP | OF | FM | GS |
|-------------|----|----|----|----|----|----|----|----|
| AC          | –  | 0.565 | 0.555 | 0.663** | 0.795*** | 0.795*** | 0.897*** | 0.900*** |
| GB          | 0.012 | –  | 0.527 | 0.643** | 0.789*** | 0.830*** | 0.974*** | 0.947*** |
| WB          | 0.012 | 0.008 | –  | 0.796*** | 0.797*** | 0.830*** | 0.949*** | 0.944*** |
| CF          | 0.052 | 0.100* | 0.137* | –  | 0.661** | 0.784*** | 0.858*** | 0.817*** |
| AP          | 0.380* | 0.470* | 0.524* | 0.242* | –  | 0.548 | 0.617* | 0.543 |
| OF          | 0.470* | 0.575* | 0.647* | 0.344* | 0.009 | –  | 0.572 | 0.591* |
| FM          | 0.558* | 0.661* | 0.751* | 0.434* | 0.045 | 0.047 | –  | 0.562* |
| GS          | 0.564* | 0.674* | 0.773* | 0.430* | 0.017 | 0.065 | 0.005 | –  |

* $P<0.05$; ** $P<0.01$; *** $P<0.001$.

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### Table 4. Analyses of molecular variance (AMOVAs) for *Exyra semicrocea* populations across the southeastern United States Coastal Plain.

| Geographic region       | Source of variation | df | Sum of squares | Variance component | % var. | $\Phi$ statistic |
|-------------------------|---------------------|----|----------------|--------------------|--------|-----------------|
| West Mississippi R.     | Between groups      | 1  | 51.275         | 1.442              | 55.21  | $\Phi_{CT}=0.552^*$ |
|                         | Among pops within groups | 1  | 3.835          | 0.615              | 23.55  | $\Phi_{SC}=0.526^{**}$ |
|                         | Within populations  | 48 | 26.619         | 0.555              | 21.24  | $\Phi_{ST}=0.788^{***}$ |
|                         |                      | 50 | 81.728         | 2.611              |        |                 |
| East Mississippi R.     | Among groups        | 2  | 88.282         | 0.826              | 46.90  | $\Phi_{CT}=0.469^{**}$ |
|                         | Among pops within groups | 3  | 5.927          | 0.013              | 0.75   | $\Phi_{SC}=0.014^{**}$ |
|                         | Within populations  | 162| 149.409        | 0.922              | 52.35  | $\Phi_{ST}=0.477^{***}$ |
|                         |                      | 169| 243.663        | 1.762              |        |                 |

* $P<0.05$; ** $P<0.01$; *** $P<0.001$.

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barrier has influenced a similar pattern in both species. Interestingly, the genetic structure of microbial communities found within the pitchers of S. alata also mirrors that of the plant [62] and, by extension, that of E. semicrocea (this study). Taken together, these patterns highlight the biological uniqueness and intimate associations among constituents of pitcher plant bog habitats in the WGCP.

In the west, the significant genetic structure among geographically close populations of E. semicrocea contrasts with an absence of genetic structure among similarly (or more widely) distanced populations in the east (Figure 2, see above). One explanation for this may be a lack of historical connectivity between patches of bogs in the west. While little is known concerning the history of pitcher plant bogs in this region, it is estimated that bogs of the WGCP may have occupied ~4,000–8,000 ha prior to European settlement [63] and been distributed among sites in as many as 20 counties (e.g., 4 parishes in Louisiana, 16 counties in Texas) [60]. However, areas that currently harbor locally abundant pitcher plant bogs are just two parishes in Louisiana (which encompasses KN) and four counties in Texas (which encompasses AN and BT). Unfortunately while historical records of small and/or rare populations can provide useful information for establishing past distributions, they may also artificially extend “known” ranges due in part to the reporting of erroneous localities, producing a false picture of historical abundance in the process [64]. Such a situation has been reported for pitcher plant records in Texas [60] and if this is the case, pitcher plant bogs in the WGCP might have been as patchy and historically isolated as they are today. Along with this, it is well known that floristic composition differs between eastern and western portions of the Coastal Plain [65,66], with these differences typically attributed to variations in soil [65] and levels of rainfall [67]. Additionally, many western bogs are supported by springs [67] rather then rainfall, the more common water source for their eastern counterparts. Such environmental differences may have contributed to habitat patchiness and the paucity of large, expansive bogs in the west relative to the east and are potential contributors to the local and significant population structure of E. semicrocea that likely extends to other obligate pitcher plant bog organisms as well.

Another explanation for the significant structure among western E. semicrocea populations is a possible difference in dispersal behavior resulting from genetic differentiation. Specifically, the COI sequence divergence between E. semicrocea from the WGCP and eastern Coastal Plain ranges from 1.9–3.0% and while sequence divergence values for Lepidoptera congeneric species pairs are generally greater than 3.0% [68], values for Lepidoptera species known to be morphologically different can be lower than 3.0% [69]. Thus, the level of difference seen here indicates a period of isolation between western and eastern E. semicrocea populations during which it is conceivable that behavioral differences may have evolved. If so, further investigations into potential morphological and behavioral differences between the eastern and western lineages of E. semicrocea are warranted since such information has important implications in future conservation efforts (see below).

Eastern population structure and Apalachicola-Chattahoochee-Flint River (ACF) break

Populations of E. semicrocea east of the Mississippi River alluvial plain are genetically structured into three distinct groups. These groups are situated within the East Gulf Coastal Plain (EGCP, USGS designation, encompassing AC/GB/WB), the South Atlantic Coastal Plain (SACP, USGS designation, encompassing AP/OF/FM/GS), and Conecuh National Forest (CF) in the proposed transition zone between the groups (although it should be noted that CF is officially within the East Gulf Coastal Plain USGS designation, Figure 1). Notably, this transition zone includes the Apalachicola-Chattahoochee-Flint (ACF) River basin,
a well-documented area for genetic discontinuity for a number of vertebrates [70], invertebrates [71], and plant [72] species. Various hypotheses on why the ACF is an area of genetic discontinuity have been proposed. One of these argues that the Apalachicola River and its resulting floodplain is one of the few systems completely bisecting the eastern Coastal Plain and therefore likely both a historical and contemporary physical barrier [73]. In addition, this system is responsible for draining the Appalachian Mountain massif, creating distinct soil differences on the resulting sides of the ACF [65]. Along with this, areas of the Coastal Plain were consistently inundated by the Gulf of Mexico during the late Pliocene and Pleistocene interglacial periods and it is thought that while these sea level increases did not completely cover the entire Coastal Plain, range fragmentations occurred due to coastal advances and the inability of endemic taxa to shift their ranges further north [73]. Under this scenario, refugia were likely to have been created both west and east of the ACF for these taxa during interglacial periods in the Pleistocene.

While a general pattern of discontinuity at the ACF basin is well established, it is not necessarily congruent across taxa. In this study, *E. semicrocea* is structured across the ACF, but with CF behaving as an outlier by possessing haplotypes commonly found both west and east of the ACF basin (Figure 2). In addition, migration estimates at CF undergo a distinct transition, suggesting the influence of a potential barrier in this area. However, the exact mechanism(s) contributing to these trends in *E. semicrocea* are difficult to identify since the region between the Mississippi and Apalachicola Rivers has a history of variable phylogeographic patterns across many species [72]. For example, studies of southeastern Coastal Plain fishes [70,74] and other vertebrate taxa [70,72] highlight some of the inconsistencies in the genetic structure of organisms from this region. Overall, this variability is likely a product of numerous interglacial events spanning the Pleistocene that resulted in refugia and divergence in isolation. Given this, future phylogeographic studies of additional taxa from this region, including *Sarracenia* species and its associated arthropods, may provide further insight toward resolving the unusual nature of this area.

With the exception of CF, the other EGCP populations (i.e., AC, GB, WB) of *E. semicrocea* exhibit demographic expansion but no genetic structure among them, even though the distances between populations are similar to those west of the Mississippi River (Figure 1). These differences are likely due to much more contiguous bog habitat, both historically and contemporarily, across the EGCP. Specifically, early writings imply a large expanse of bog, possibly stretching from Pensacola, Florida, west to Pascagoula, Mississippi (a span of 130 km), before the late 1800s [75,76]. Support for this comes from habitat suitability assessments and soil surveys of the EGCP indicating pitcher plant bogs may have occupied 293,500 ha across the region in pre-Columbian times [65]. In contrast to the EGCP, populations of *E. semicrocea* in the SACP (i.e., AP, OF, FM, GS) exhibited in some cases weak, but significant, genetic structure (Table 3). One potential driver for this pattern might again be habitat patchiness. For example, sites such as OF and FM have minimal pitcher plant bog habitat due to large swaths of historic swampy areas. Furthermore, in the northern portions of the SACP (i.e., South and North Carolina), regionally specific “bog-like” habitats known as pocosins and Carolina Bays either exclude (i.e., pocosin) or create elliptical, irregular pitcher plant habitats (i.e., Carolina Bays) [65]. These alternative habitat types as well as the effects of glacial advances and retreats over time in this region may contributed to the generation of pitcher plant bogs with patchy distributions and initiated the process of differentiation among *E. semicrocea* populations in the SACP.

**Conservation Implications**

As mentioned previously, pitcher plant bogs of the southeastern United States Coastal Plain possess the most diverse assemblages of carnivorous plants in the world [13] while also belonging to one of the most critically endangered ecosystems in North America [10]. Thus, it is imperative to further develop sound management practices for maintaining the health and integrity of the plants and associated animal communities in those habitats that remain. Established techniques toward this end are prescribed burns implemented by state and US Federal agencies. In contrast to the historical and natural burning that occurred every one to ten years during the growing season [77], these regular, controlled burns are often conducted in the winter (i.e., non-growing season). While it is assumed that the endemic members of this community, such as *E. semicrocea*, are most likely fire adapted [70], it remains unclear how these unnatural winter burns may impact the overall composition of pitcher plant bog communities. However, detection of high mortality among larvae with even a low intensity, patchy fire and further observations of *E. semicrocea* overwintering only as 4th or 5th instar larva [78] suggest winter burns are potentially detrimental to local populations. For example, *E. semicrocea* was not found at four sites (i.e., Centre, AL; Prattville, AL; Reed Branch, GA and Eller Seep, NC) visited for this study in spite of previously being reported as occurring at these locations (pers. comm. D. Folkerts and M. Hodges). We propose that previous winter burns conducted at these four sites in conjunction with isolation and fragmentation may have contributed to the potential extirpation of their *E. semicrocea* populations. This is alarming as there are other endemic pitcher plant arthropods that are considerably less common, and with potentially weaker dispersal capabilities, that may also be adversely impacted by such management techniques. This highlights the need for additional research specifically addressing the effects of fire on other constituents of pitcher plant bog communities and in the interim and until more information is gathered, it may be prudent to maintain unburned habitat during prescribed fire events (preferably during the growing season) as refugia for community members susceptible to such management practices.

Although pitcher plants have received attention with several species protected and others listed as endangered, threatened or of special concern, their arthropod inhabitants have received little or no conservation consideration. Because many of these arthropods are obligate associates of pitcher plants, they have likely suffered equivalent declines. Despite the fact that none have official protection status and the roles and importance that these endemic arthropods play within these habitats are poorly understood, conservation efforts should ideally strive to protect all components of threatened ecosystems. Due to the obvious effects that *E. semicrocea* has on *Sarracenia* spp. (i.e., leaf herbivory), this species has been one of the more studied [24,25,27]. Thus, the identification here of *E. semicrocea* as encompassing six distinct population groups within two divergent lineages (i.e., western and eastern) is of importance and should be considered when developing management and conservation strategies for pitcher plant bogs in the southeastern United States Coastal Plain. In this context, the three populations of *E. semicrocea* west of the Mississippi River (KN, AN, BT) are distinctive due to their strong genetic structure and this is further supported by the comparative phylogeographies of *E. semicrocea*, *S. alata*, and the microbial community within the pitchers representing unique and coevolving units in the WGCP (see above). Thus, steps should be taken to maintain the distinct genetic diversity at each of these sites. Similarly, the three population groups (i.e., EGCP, CF, SACP) of *E. semicrocea* in the eastern portion of its range deserve comparable attention. Overall,
the two *E. semicoccea* lineages (i.e., west and east) could minimally be considered Evolutionary Significant Units (ESUs) of the species. The recognition of ESUs, defined as one or a set of populations with a distinct evolutionary heritage, can aid managers in prioritizing sites and activities related to conservation [79]. This region of the southeastern Coastal Plain has the highest biodiversity in the United States [80] and therefore future protection and/or restoration of this area has further implications for not only pitcher plant bogs and their constituents, but also other taxa [81,82] and unique habitats [83] as well.

Supporting Information

**Table S1** Distributions of other taxa [81,82] and unique habitats [83] as well.

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**Author Contributions**

Conceived and designed the experiments: JDS SRS DRF. Performed the experiments: JDS. Analyzed the data: JDS. Contributed reagents/materials/analysis tools: SRS. Wrote the paper: JDS SRS DRF.

References

1. Wilson EO (1988) Biodiversity. Washington: National Academy Press.
2. Worm B, Barbier EB, Beaumont N, Duffy J, Folke C, et al. (2006) Impacts of biodiversity loss on ocean ecosystem service. Science 314: 787–790.
3. Moyle PB, Williams JE (1990) Biodiversity loss in the temperate zone: decline of the native fish fauna of California. Conservation Biology 4: 475–484.
4. Norse EA (1990) Ancient forests of the Pacific Northwest. Washington: The Wilderness Society and Island Press.
5. Dahlem GA, Nacci RFC (2006) Flesh flies (Diptera: Sarcophagidae) associated with North American pitcher plants (Sarraceniaceae), with descriptions of three new species. Annuals Entomological Society of America 99: 218–240.
6. Wilson EO (1985) The biological diversity crisis. BioScience 35: 700–706.
7. Moyle PB, Williams JE (1990) Biodiversity loss in the temperate zone: decline of the native fish fauna of California. Conservation Biology 4: 475–484.
8. Norse ME (1991) Conservation: tactics for a constant crisis. Science 253: 744–750.
9. Wilson EO (1983) The biological diversity crisis. BioScience 33: 700–706.
10. Noss RF, LaRoe III ET, Scott JM (1995) Endangered ecosystems of the United States: a preliminary assessment of loss and degradation. National Biological Service Biological Report 28. U.S. Department of the Interior, Washington, D.C.
11. Noss RF (1989) Longleaf pine and wiregrass: keystone components of an endangered ecosystem. Natural Areas Journal 9: 211–213.
12. Folkerts GW, Folkerts DR (1993) Southeastern pitcher plant bogs: A natural history sketch. Tipularia 8: 10–16.
13. Folkerts DR (1999) Pitcher plant wetlands of the southeastern United States: Arthropod associates. In: Batzer DP, Radar RR, Wissinger SA, eds Invertebrates in Freshwater Wetlands of North America. New York: Ecology and Management, Wiley, pp 247–273.
14. USDA, NRCS (2009) The PLANTS Database, National Plant Data Center. Baton Rouge, LA 70874-4490 USA. Available: http://plants.usda.gov. Accessed 2009 October 22.
15. Brewer JS, Baker DJ, Niero AS, Patterson AL, Roberts RS, et al. (2011) Carnivory in plants as a beneficial trait in wetlands. Aquatic Botany 94: 62–70.
16. Ellison AM, Gotelli NJ (2009) Energetics and the evolution of carnivorous plants-Darwin’s ‘most wonderful plants in the world’. Journal of Experimental Botany 60: 19–42.
17. Givnish TJ, Burkhardt EL, Happel RE, Weinstein JD (1984) Carnivory in the bromeliad Baccharis reducta, with a cost-benefit model for the general restriction of carnivorous plants to sunny, moist, nutrient-poor habitats. American Naturalist 124: 479–497.
18. Darwin C (1875) Insectivorous Plants. London: Murray.
19. Givnish TJ, Burkhardt EL, Happel RE, Weinstein JD (1984) Carnivory in the bromeliad Baccharis reducta, with a cost-benefit model for the general restriction of carnivorous plants to sunny, moist, nutrient-poor habitats. American Naturalist 124: 479–497.
20. Godt MJW, Hamrick JL (1996) Genetic structure of two endangered pitcher plants, *Sarracenia flava* and *Sarracenia alata*. American Journal of Botany 83: 1016–1023.
21. Godt MJW, Hamrick JL (1998) Allelozyme diversity in the endangered pitcher plant *Sarracenia rubra* ssp. *alabamensis* (Sarraceniaceae) and its close relative *S. rubra* ssp. *rubra*. American Journal of Botany 85: 802–810.
22. Wang ZE, Hamrick JL, Godt MJW (2004) High genetic diversity in *Sarracenia leucophylla* (Sarraceniaceae), a carnivorous wetland herb. Journal of Heredity 95: 233–244.
23. Koopman MM, Caresta BC (2010) Conservation genetic inferences in the carnivorous pitcher plant *Sarracenia alata* (Sarraceniaceae). Conservation Genetics 11: 2007–2038.
24. Rymal DE, Folkerts GW (1982) Insects associated with pitcher plants (Sarracenia Sarraceniaceae), and their relationship to pitcher plant conservation: A review. Journal of the Alabama Academy of Science 53: 151–151.
25. Folkerts DR, Folkerts GW (1996) Aliases for field identification of pitcher plant moths of the genus *Exyra* (Lepidoptera: Noctuidae). Environ Entomol 25: 137–137.
26. Lafontaine JD, Poole RW (1991) Noctuoidae, Noctuidae (Part). In: Domnick RB, et al. (1991) The Moths of America north of Mexico, fascicle 25:1. Washington: The Wedge Entomological Research Foundation.
27. Jones FM (1921) Pitcher plants and their moths. Natural History 21: 297–316.
28. Coffroth M, Lasker HR, Diamond ME, Bruenn JA, Berrema I (1992) DNA fingerprinting of a gorgonian coral: a method for detecting clonal structure in a vegetative species. Marine Biology 114: 317–325.
29. Folmer O, Black M, Hoeh W, Lutz R, Vrijenhoek R (1994) DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. Molecular Marine Biology and Biotechnology 3: 294–299.
30. Avise JC, Arnold J, Ball RM, Berrema I, Lamb T, et al. (1987) Intraspecific phylogeny: the mitochondrial DNA bridge between population genetics and systematics. Annual Review of Ecology and Systematics 18: 489–522.
31. Meng XF, Shi M, Chen XX (2008) Population genetic structure of *Chiropalaceus* (Walker) (Lepidoptera: Crambidae): strong subdivision in China inferred from microsatellite markers and mtDNA gene sequences. Molecular Ecology 17: 2800–2897.
32. Koebiranz RL, Lopez JD, Loera J, Hendricks DE (1994) Limited mitochondrial DNA polymorphism in two North American populations of *Helithis micros* (Lepidoptera: Noctuidae). Annals of the Entomological Society of America 87: 856–866.
33. Nei M (1987) Molecular Evolutionary Genetics. New York: Columbia University Press.
34. Rozas J, Sanchez-DelBarrio JC, Messegue X, Rozas R (2003) DnaSP, DNA polymorphism analyses by the coalescent and other methods. Bioinformatics 19: 2496–2497.
35. Hudson RR, Boos DD, Kaplan NL (1992) A statistical test for detecting geographic subdivision. Molecular Biology and Evolution 9: 138–151.
36. Hudson RR (2000) A new statistic for detecting genetic differentiation. Genetics 155: 2011–2014.
37. Excoffer L, Laval G, Schneider S (2005) Arlequin ver. 3.0: An integrated software package for population genetics data analysis. Evolutionary Bioinformatics Online 1: 47–50.
38. Excoffer L, Heckel G (2006) Computer programs for population genetics data analysis: a survival guide. Nature Reviews Genetics 7: 743–758.
39. Excoffer L, Smouse PE, Quattro JM (1992) Analysis of molecular variance inferred from metric distances among DNA haplotypes: Application to human mitochondrial restriction data. Genetics 131: 479–491.
40. Tamura K, Nei M (1987) Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. Molecular Biological and Evolution 9: 512–526.
44. Okansen J, Blanchet F, Kandi R, Legrande P, O’Hara RB, et al. (2010) vegan: Community ecology package. Available: http://vegan.r-forge.r-project.org.
45. R Development Core Team (2008) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. Available: http://www.R-project.org
46. Nielsen R, Wakeley J (2001) Distinguishing migration from isolation: a Markov chain Monte Carlo approach. Genetics 156: 888–896.
47. Templeton AR, Crandall KA, Sing CF (1992) A cladistic analysis of phenotypic associations with haplotypes inferred from restriction endonuclease mapping and DNA sequence data. III. Cladogram estimation. Genetics 131: 619–633.
48. Clement M, Posada D, Crandall KA (2000) TCS: a computer program to estimate gene genealogies. Molecular Ecology 9: 1657–1659.
49. Tajima F (1989) Statistical method for testing the neutral mutation hypothesis by DNA Polymorphism. Genetics 123: 585–595.
50. Templeton AR, Crandall KA, Sing CF (1992) A cladistic analysis of phenotypic associations with haplotypes inferred from restriction endonuclease mapping and DNA sequence data. III. Cladogram estimation. Genetics 131: 619–633.
51. Fu YX (1997) Statistical tests of neutrality of mutations against population growth, hitchhiking and background selection. Genetics 147: 915–925.
52. Akey JM, Eberle MA, Rieder MJ, Carlson CS, Shriver MD, et al. (2004) Population history and natural selection shape patterns of genetic variation in 132 genes. Public Library of Science 2: 257–279.
53. Bridges EL, Orzell SL (1989) Longleaf pine communities of the West Gulf coastal plain. Natural Areas Journal 9: 246–263.
54. Oksanen J, Blanchet F, Kindt R, Legendre P, O’Hara RB, et al. (2010) vegan: Community ecology package. Available: http://vegan.r-forge.r-project.org.
55. MacRoberts MH, MacRoberts BR (2001) Bog communities of the West Gulf Coastal Plain: a profile. Bog Research Papers in Botany and Ecology 1: 1–151.
56. Miller BP, Earight NJ, Lamont BB (2007) Record error and range contraction, real and imagined, in the restricted shrub Banksia hookeriana in south-western Australia. Diversity and Distribution 13: 406–417.
57. Brower AVZ (1994) Rapid morphological radiation and convergence among pitcher plants. Ecology Letters. In press.
58. Folkerts GW (1982) The Gulf Coast pitcher plant bogs. American Scientist 70: 261–267.
59. Folkerts GW (1991) A preliminary classification of pitcher plants habitats in the southeastern United States. Journal of the Alabama Academy of Science 62: 199–225.
60. Folkerts GW (1991) A preliminary classification of pitcher plants habitats in the southeastern United States. Journal of the Alabama Academy of Science 62: 199–225.
61. Folkerts GW, Wynne RA, Bundsen RL, Keddy PA (1994) Phylogeography of the Northern short-tailed shrew, Blarina brevicauda (Insectivora: Soricidae): past fragmentation and postglacial recolonization. Molecular Ecology 12: 1435–1449.
62. MacRoberts MH, MacRoberts BR (2001) Bog communities of the West Gulf Coastal Plain: a profile. Bog Research Papers in Botany and Ecology 1: 1–151.
63. Miller BP, Earright NJ, Lamont BB (2007) Record error and range contraction, real and imagined, in the restricted shrub Banksia hookeriana in south-western Australia. Diversity and Distribution 13: 406–417.
64. Bolger AVZ, Brower AVZ (1994) Rapid morphological radiation and convergence among pitcher plants. Ecology Letters. In press.
65. Folkerts GW (1982) The Gulf Coast pitcher plant bogs. American Scientist 70: 261–267.
66. Folkerts GW (1991) A preliminary classification of pitcher plants habitats in the southeastern United States. Journal of the Alabama Academy of Science 62: 199–225.
67. Bridges EL, Orzell SL (1989) Longleaf pine communities of the West Gulf coastal plain. Natural Areas Journal 9: 246–263.
68. McRoberts MH, MacRoberts BR (2001) Bog communities of the West Gulf Coastal Plain: a profile. Bog Research Papers in Botany and Ecology 1: 1–151.
69. Miller BP, Earright NJ, Lamont BB (2007) Record error and range contraction, real and imagined, in the restricted shrub Banksia hookeriana in south-western Australia. Diversity and Distribution 13: 406–417.
70. Folkerts GW (1982) The Gulf Coast pitcher plant bogs. American Scientist 70: 261–267.
71. Folkerts GW (1991) A preliminary classification of pitcher plants habitats in the southeastern United States. Journal of the Alabama Academy of Science 62: 199–225.
72. Bridges EL, Orzell SL (1989) Longleaf pine communities of the West Gulf coastal plain. Natural Areas Journal 9: 246–263.
73. Avise JC (1996) Towards a regional conservation genetic perspective: Phylogeography of faunas in the southeastern United States. In: Avise JC, Hamrick JL, eds. Conservation Genetics; Case Histories from Nature. New York: Chapman & Hall, pp. 451–470.
74. Avise JC (1996) Towards a regional conservation genetic perspective: Phylogeography of faunas in the southeastern United States. In: Avise JC, Hamrick JL, eds. Conservation Genetics; Case Histories from Nature. New York: Chapman & Hall, pp. 451–470.
75. Bartram W (1791) Travels through North and South Carolina, Georgia, east and west Florida, etc., Facsimile ed. New York: Dover Publishing, Inc.
76. Bartram W (1791) Travels through North and South Carolina, Georgia, east and west Florida, etc., Facsimile ed. New York: Dover Publishing, Inc.
77. Chapman HH (1932) Is the longleaf type a climax? Ecology 13: 328–334.
78. Chapman HH (1932) Is the longleaf type a climax? Ecology 13: 328–334.
79. Chapman HH (1932) Is the longleaf type a climax? Ecology 13: 328–334.
80. Avise JC (1996) Towards a regional conservation genetic perspective: Phylogeography of faunas in the southeastern United States. In: Avise JC, Hamrick JL, eds. Conservation Genetics; Case Histories from Nature. New York: Chapman & Hall, pp. 451–470.
81. Keddy PA (2009) Thinking big: a conservation vision for the southeastern United States: the flatwoods salamander, Ambystoma cingulatum, as a test case. Molecular Ecology 16: 415–429.
82. Walters J, Doerr P, Carter III J (1988) The cooperative breeding system of the red-cockaded woodpecker. Ethology 78: 275–305.
83. Folke CR, Folke GW, Folke DR (2011) A floristic study of a steephead stream in northwestern Florida. Southeastern Naturalist 10: 289–302.