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Population Genetics of the Burrowing Mayfly *Dolania americana:* Geographic Variation and the Presence of a Cryptic Species

by

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The genetic structure of *Dolania* populations in South Carolina, Alabama, and Florida was examined using starch gel electrophoresis. All study populations except Alabama consisted entirely of *D. americana* Edmunds and Traver. Electrophoresis revealed that the Alabama site contained both *D. americana* and an undescribed species (*D.* sp. nov.), with the latter taxon predominating. Individuals of the two taxa had nearly fixed allelic differences at two loci (*Est 4* and *Gda*). The extent of genetic differentiation between populations of the two taxa in the same river or between adjacent river systems was significantly greater than between *D. americana* populations separated by large geographic distances. Populations of both species were characterized by relatively normal levels of polymorphic loci (average = 23%) but unusually low levels of heterozygosity (average = 0.03). Genetic variation between the Florida and South Carolina populations of *D. americana* was statistically significant. There was also some indication of slight genetic differentiation among populations of *D. americana* within the Blackwater River in Florida. No significant genetic variation was observed between cohorts of *D. americana* at a given site.

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INTRODUCTION

Dolania americana Edmunds and Traver is a burrowing mayfly whose predaceous larvae live in the shifting sandy bottom of coastal plain streams from North Carolina to Louisiana (see Peters et al., 1987 for review). The species is reported to have a two year life cycle in South Carolina (Harvey et al., 1980) and in northern Florida (Peters et al., 1987). All populations are characterized by an unusually short reproductive period on both a diel and seasonal basis (Peters and Peters, 1977; Sweeney and Vannote, 1982). For example, practically all larvae in a given population undergo metamorphosis within a 7-10 day period in late spring and, on a given day, the winged stage lasts only about 10 to 30 minutes. The brevity of the adult reproductive period seems to be a life history adaptation related, in part, to reducing predation (Sweeney and Vannote, 1982).

Here we examine the genetic structure of populations of *D. americana* in South Carolina, Alabama, and Florida. Our working hypothesis for the study was that

the extremely brief winged stage might severely limit geographic dispersal in the species and result in a nonpanmictic population structure and substantial geographic differentiation at the allozyme level. Since the species has a two year life cycle, we also examined year to year genetic variation for two populations.

METHODS

Adult *D. americana* specimens were collected from the field at three locations in 1986: SC1 = the type locality of *D. americana* at Upper Three Runs, Aiken County, South Carolina (33°23'N; 81°37'W), FL1 = Blackwater River, Okaloosa County, Florida (30°59'N; 86°43'W), and FL2 = Blackwater River, Okaloosa County, Florida (30°44'N; 86°47'W). Additional specimens were obtained from sites FL1 and FL2 in 1987 as well as from a fourth site: AL1 = Yellow River, Covington County, Alabama (31°00'N; 86°32'W). For sites FL1 and AL1 in 1987, mature larvae were collected from the site and reared to the adult in the laboratory. Adults were collected from the field at site FL2 in 1987. All adults were frozen shortly after collection and shipped to the Stroud Center in Avondale, Pennsylvania, where they were stored in an ultra low freezer (-80°C) until analysis.

Specimens were electrophoresed in 1986 and 1987 on horizontal starch gels as described in Sweeney et al. 1987. The 1986 and 1987 data from the FL1 and FL2 sites have been presented separately for comparative purposes. It should be noted that we were able to score more loci per individual specimen during electrophoresis experiments in 1987 than in 1986 due to new and/or improved techniques. In order to facilitate comparison among all study populations using the maximum number of loci, we repeated electrophoresis experiments in 1987 on 18 additional specimens that had been collected from site SC1. Data analysis was performed with the BIOSYS-1 program (version 1.6; Swofford and Selander, 1981). The significance of F statistics (Wright, 1978) was tested using chi-square analysis (Baker, 1981).

RESULTS

Large differences in allele frequencies at two loci *(Gda, Est 4)* strongly suggest that the *Dolania* population at the Yellow River, Alabama site (AL1) consists of two taxa: an undescribed species (*D.* sp. nov.) which is relatively abundant and the rare *D. americana* (Table 1). Specifically, the common allele(s) for both *Gda* and *Est 4* in the *D.* sp. nov. population were either extremely rare or absent from all the other populations. Although differences are not completely fixed for the two loci, they are large enough to effectively argue genetic isolation of *D.* sp. nov. and *D. americana.* Figure 1 illustrates the 13 two-locus *(Gda, Est 4)* genotypes we observed in *Dolania* species, and the number of times each was observed.

Table 1. Allele frequencies for 14 polymorphic enzyme loci in *Dolania*. The following loci were monomorphic: MDH1, MDH2, SOD2, AO, ME, G6PDH, 6PGD, LDH, ADK, GDH, ISDH2, aGPDH, G3PDH, AAT2, EST1, EST2, EST5, EST6. The full name and Enzyme Commission number for each enzyme code is listed in Sweeney et al. (1987).

Enzyme code		*D. americana* SC1-86	FL1-86	FL1-87	FL2-86	FL2-87	AL1-87	*D* sp. nov. AL1-87
SOD1	(N)	63	24	35	40	40	2	22
	A	0.02	0.06	0.01	0.03	0.01	–	–
	B	0.98	0.94	0.99	0.98	0.99	1.00	1.00
GPI	(N)	63	24	35	40	40	2	22
	A	0.02	–	–	–	–	–	–
	B	0.74	0.92	0.86	0.90	0.88	0.75	1.00
	C	–	–	0.04	0.01	–	0.25	–
	D	–	0.02	–	–	–	–	–
	E	0.25	0.06	0.10	0.09	0.13	–	–
ALD	(N)	63	24	35	40	40	2	22
	A	1.00	1.00	1.00	1.00	1.00	1.00	0.93
	B	–	–	–	–	–	–	0.07
PGM	(N)	63	24	35	40	40	2	22
	A	–	–	0.01	0.03	0.01	–	–
	B	1.00	0.98	0.97	0.98	0.95	1.00	0.96
	C	–	0.02	0.01	–	0.04	–	0.05
HEX	(N)	63	24	35	40	40	2	22
	A	1.00	0.88	0.86	0.95	0.99	1.00	1.00
	B	–	0.13	0.14	0.05	0.01	–	–
ISDH 1	(N)	63	24	35	40	40	2	22
	A	1.00	1.00	1.00	1.00	1.00	1.00	0.96
	B	–	–	–	–	–	–	0.05
AAT1	(N)	63	24	35	40	40	2	22
	A	–	0.02	–	–	0.05	–	–
	B	1.00	0.98	1.00	1.00	0.95	1.00	1.00
LAP1	(N)	63	24	35	40	40	2	22
	A	1.00	0.98	0.96	0.98	0.96	1.00	0.96
	B	–	0.02	0.04	0.03	0.04	–	0.05
LAP2	(N)	63	24	35	40	40	2	22
	A	1.00	0.98	0.94	1.00	1.00	1.00	1.00
	B	–	0.02	0.06	–	–	–	–
MPI	(N)	63	24	35	40	40	2	22
	A	–	0.04	0.01	0.03	0.01	–	–
	B	1.00	0.94	0.97	0.96	0.99	1.00	1.00
	C	–	0.02	0.01	0.01	–	–	–
EST4	(N)	63	24	35	40	40	2	22
	A	0.01	–	–	–	–	–	–
	B	0.25	0.15	0.11	0.14	0.04	–	–
	C	0.46	0.85	0.81	0.78	0.83	1.00	0.02
	D	–	–	0.01	–	0.01	–	0.25
	E	0.28	–	0.06	0.09	0.13	–	0.02
	F	–	–	–	–	–	–	0.71

Table 1 (cont.)

Enzyme code		*D. americana* SC1-86	FL1-86	FL1-87	FL2-86	FL2-87	AL1-87	*D* sp. nov. AL1-87
GDA	(N)	18	0	35	0	40	2	22
	A	1.00		1.00		1.00	1.00	0.02
	B	–		–		–	–	0.96
	C	–		–		–	–	0.02
ACON1	(N)	18	0	35	0	40	2	22
	A	0.03		0		–	–	–
	B	0.97		1.00		1.00	1.00	0.96
	C	0		0		–	–	0.05
ACON2	(N)	18	0	35	0	40	2	22
	A	–		–		–	–	0.05
	B	0.97		1.00		1.00	1.00	0.96
	C	0.03		–		–	–	–

The difference between species at *Gda* was nearly fixed, with a single individual of *D.* sp. nov. heterozygous for the common *D.* sp. nov. allele (B) and the common *D. americana* allele (A). This individual was heterozygous for the two common *D.* sp. nov. alleles at *Est 4*. One of these alleles (D) also occurred in *D. americana,* but only rarely (frequency = 0.01), so the probability that this individual represents an F_1 hybrid is extremely small. Although the allele frequencies we observed do not exclude the possibility of hybridization, it would be very rare at best.

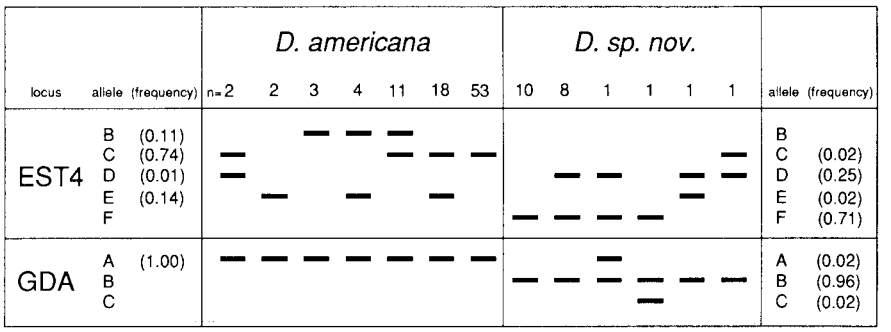

Fig. 1. Banding patterns for the 13 two-locus *(Est 4* and *Gda)* genotypes of esterase *(Est 4)* and guanine deaminase *(Gda)* observed in *Dolania* species. Each column represents a particular genotype, with n indicating the number of individuals observed for that genotype at the top. Frequencies for each allele are given in parentheses by species.

Two individuals collected at AL1 were clearly *D. americana* according to their *Gda, Est 4* genotype (i.e., homozygous for allele A at *Gda* and homozygous for allele C at *Est 4*). We excluded them from the data set and, for analytical purposes, have treated the AL1 populations as containing only *D.* sp. nov. individuals.

The proportion of loci found to be polymorphic averaged about 23% for both *D. americana* and *D.* sp. nov. (Table 2) and was within the range of levels found in other mayfly populations in eastern North America (Sweeney et al., 1987). In contrast, both observed and expected heterozygosities (avg: 0.03) were lower than for all but one of the 18 species of mayflies reported to date (Sweeney et al., 1987, Funk et al., 1988).

Wright's (1978) F_{st} values, which measure genetic differentiation among populations, were calculated for each polymorphic locus using two data sets: one included only the three *D. americana* populations (SC1, FL1-87, FL2-87) while the other set also included the *D.* sp. nov. population (SC1, FL1-87, FL2-87, AL1). For the three *D. americana* populations, the average F_{st} value for all loci was 0.059 (range: 0.000 to 0.097) with 5 of 11 loci indicating significant differentiation (Table 3). Adding the *D.* sp. nov. population increased the mean F_{st} from 0.059 to 0.364 with 10 of 14 loci being statistically significant.

Table 2. Summary statistics [mean (S.E.)] of allele frequency data for geographic populations of *Dolania* mayflies.

Species	Population	Number of Loci Examined	Mean Sample Size Per Locus	Mean No. of Alleles Per Locus	Percentage of Loci Polymorphic[a]	Mean Heterozygosity	
						Direct-Count	Hardy-Weinberg Expected[b]
D. americana	SC1-86	34	56.4 (2.8)	1.2 (0.1)	14.7	0.036 (0.023)	0.035 (0.022)
D. americana	FL1-86	29	24.0 (0.0)	1.4 (0.1)	31.0	0.030 (0.010)	0.036 (0.013)
D. americana	FL1-87	34	35.0 (0.0)	1.4 (0.1)	23.5	0.032 (0.013)	0.034 (0.014)
D. americana	FL2-86	29	40.0 (0.0)	1.3 (0.1)	24.1	0.024 (.010)	0.030 (0.015)
D. americana	FL2-87	34	40.0 (0.0)	1.3 (0.1)	23.5	0.026 (0.012)	0.026 (0.011)
D. americana	all pops	32	39.1 (5.2)[c]	1.3 (0.0)[c]	23.4	0.030 (0.002)[c]	0.032 (0.002)[c]
D. sp. nov.	AL1-87	34	22.0 (0.0)	1.3 (0.1)	23.5	0.035 (0.016)	0.033 (0.014)

[a] A locus is considered polymorphic if more than one allele was detected
[b] Unbiased estimate (see Nei 1978)
[c] Standard error of the mean values for each population

Table 3. A comparison of Wright's (1978) F statistics calculated from allele frequency data for *D. americana* (sites SC1, FL1-87, FL2-87) and for a mixture of *D. americana* and *D.* sp. nov. (sites SC1, FL1-87, FL2-87, AL1).

	D. americana and *D.* sp. nov.				*D. americana*			
LOCUS	F_{is}	F_{it}	F_{st}	$\chi^2(F_{st})$	F_{is}	F_{it}	F_{st}	$\chi^2(F_{st})$
SOD1	–0.015	–0.011	0.004	1.280	–0.015	–0.014	0.000	0.000
GPI	–0.153	–0.071	0.071	22.720*	–0.153	–0.121	0.028	7.728*
ALD	–0.073	–0.017	0.052	16.640*				
PGM	–0.039	–0.026	0.013	4.160	–0.035	–0.021	0.014	3.864
HEX	0.059	0.151	0.097	31.040*	0.059	0.139	0.085	23.460*
ISDH1	–0.048	–0.011	0.034	10.880*				
AAT1	–0.053	–0.013	0.038	12.160*	–0.053	–0.017	0.034	9.384*
LAP1	–0.044	–0.032	0.011	3.520	–0.042	–0.028	0.014	3.864
LAP2	–0.061	–0.014	0.043	13.760*	–0.061	–0.019	0.039	10.764*
MPI	–0.019	–0.008	0.011	3.520	–0.019	–0.011	0.008	2.208
EST4	0.029	0.369	0.350	112.000*	0.086	0.174	0.097	26.772*
GDA	–0.035	0.939	0.941	301.120*				
ACON1	–0.040	–0.014	0.025	8.000*	–0.029	–0.009	0.019	5.244
ACON2	–0.040	–0.014	0.025	8.000*	–0.029	–0.009	0.019	5.244
MEAN	–0.030	0.345	0.364	116.480*	–0.012	0.048	0.059	16.284*

* $p \leq 0.05$

Table 4. Average Nei (1978) genetic distance (above diagonal) and Rogers (1972) genetic distance (below diagonal) among various cohorts, populations, and species of *Dolania*.

	POPULATION	1	2	3	4	5	6
1	FL2-86 *(D. americana)*	****	0.004	0.001	0.001	0.000	0.021
2	SC1-86 *(D. americana)*	0.020	****	0.006	0.005	0.004	0.019
3	FL1-86 *(D. americana)*	0.011	0.029	****	0.000	0.001	0.024
4	FL1-87 *(D. americana)*	0.010	0.026	0.010	****	0.001	0.023
5	FL2-87 *(D. americana)*	0.010	0.021	0.016	0.014	****	0.023
6	AL1-87 *(D.* sp. nov.)	0.039	0.039	0.046	0.044	0.039	****

The significance of the genetic difference between *D. americana* and *D.* sp. nov. populations is further revealed by Nei's (1978) genetic distance values (Table 4). These data, which do not include the *Gda* locus because it was not scored for FL1 or FL2 in 1986, clearly show that intraspecific genetic differences were zero for *D. americana* from year to year at a given site and low between adjacent (FL1, FL2) or distant (FL1 and FL2 vs. SC1) populations. In contrast, genetic distances were substantially higher between the *D.* sp. nov. populations at site AL1 and any of the *D. americana* populations. A phenogram depicting Nei's (1978) genetic similarity between the *Dolania* populations at the four principal study sites, which includes *Gda* information, further supports the interpretation of a cryptic species at site AL1 (Fig. 2). Thus, the number of genes shared by

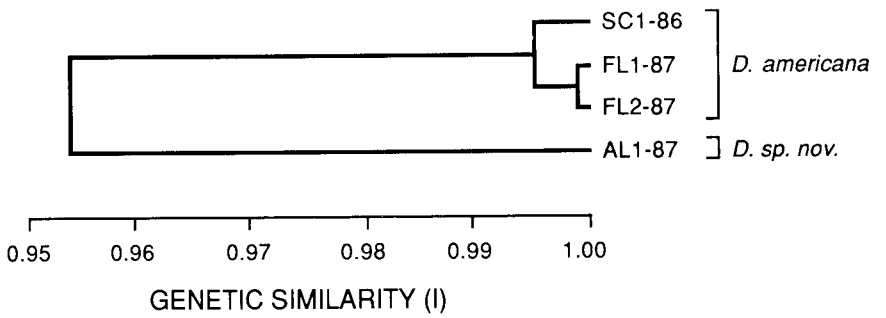

Fig. 2. Phenogram of four populations of *Dolania* based on a cluster analysis (UPGMA) of Nei's (1978) Genetic Identity (I) values.

Dolania populations in adjacent drainages and separated by only about 16 km (eg. FL1 or FL2 vs. AL1) appears to be substantially lower than the number shared by populations separated by about 480 km.

DISCUSSION

Interspecific variation

Our study has revealed highly significant genetic differentiation between *Dolania* populations in northern Florida (FL1 and FL2) and certain individuals in the population in Alabama (AL1). The observation of nearly fixed differences in allele frequencies at two loci between the Florida and Alabama populations strongly suggests that little, if any, recent gene flow has occurred between them. This interpretation is reinforced by the low levels of genetic differentiation between the Florida and South Carolina populations relative to differences between the Florida and Alabama populations. The information, in addition to the co-occurrence of a few individuals at the Alabama site that appear to be genetically very similar to the Florida populations, leads us to conclude firmly that: (1) two *Dolania* taxa presently occur in the southeastern region of North America (*viz. D. americana* and *D.* sp. nov.); and (2) the Alabama study site consists of two co-occurring *Dolania* species with *D.* sp. nov. predominating.

The following arguments can be made against the validity of our conclusions but we largely view them as frivolous. First, the genetic similarity between populations of the two taxa (as we presently view them) averaged about 0.95 (Fig. 2). Although this is substantially lower than the genetic similarity between conspecific populations of *D. americana,* it is not as low as the average genetic similarity values that have been observed between sibling species of most terrestrial insects ($\bar{x}$ = 0.78; as calculated from Pashley, 1983 and Brussard et al., 1985) or other mayflies ($\bar{x}$ = 0.75 as calculated from Saura et al., 1979, Zurwerra et al., 1986, 1987, Sweeney et al., 1987, Funk et al., 1988). Furthermore, the

genetic similarity between the two *Dolania* taxa was about the same as those observed for subspecies of other terrestrial insects ($\bar{x}$ = 0.90; Pashley, 1983 and Brussard et al., 1985).

We counter these arguments by noting that heterozygosities were extremely low in *Dolania* relative to other insects and, despite nearly fixed differences at two loci, the relatively low gene diversity among the remaining 30 or so loci tended to increase our estimate of overall genetic similarity. More importantly, we are not aware of any instance where subspecies designations were assigned when fixed or nearly fixed differences at two or more loci were observed in sympatric populations regardless of overall genetic similarity.

A second argument against our conclusions is that although allele differences at two critical loci are nearly fixed, we have not demonstrated complete fixation. Thus, we cannot absolutely rule out recent gene flow (albeit at low levels) between the populations and, by definition, have no basis for considering them to be separate, reproductively isolated species. We note, however, that inspection of individual genotypes for the *Est 4* and *Gda* loci revealed only a single individual posessing alleles found in both species. This individual (which we consider *D.* sp. nov.) was heterozygous for the common alleles of each species at *Gda,* but the *Est 4* allele that could have come from *D. americana* is in fact quite rare in that species (frequency = 0.01), but relatively common in *D.* sp. nov. (frequency = 0.25). The possibility that this individual was an F_1 hybrid is therefore remote, and we conclude that gene flow between the two taxa is extremely low (if it occurs at all).

A third argument against our interpretation is that we only sampled a small portion of the genome of these taxa and it is well documented that enzyme electrophoresis does not consistently reveal all the genetic variation that is actually present. The former point is true since only 32 loci were examined but this sample size is, on the average, about twice as high as most other published insect studies (except *Drosophila;* see Graur, 1985 for recent review). It is also true that inadequacies in the electrophoretic technique may have failed to resolve certain differences between *Dolania* populations that might shed a different interpretation. However, observed differences in mobility of the magnitude reported for the *Est 4* and *Gda* loci are rarely, if ever, questioned with regard to indicating significant differences in protein configuration and hence underlying genetic structure.

Intraspecific variation

Levels of gene diversity (heterozygosity) for *Dolania* populations were extremely low relative to other mayflies (Sweeney et al., 1987, Funk et al., 1988) and insects in general (Graur, 1985). Heterozygosity levels vary substantially among various major groups of organisms (Nevo et al., 1984) as well as within specific groups (Wooten and Smith, 1985, Graur, 1985). For insects, considerable attention and debate has focused on the low gene diversity of Hymenoptera (Berkelhamer, 1983, Graur, 1985, Reeve et al., 1985, Owen, 1985), which now

appears to be related to the high degree of sociality in the order and its importance in reducing effective population size. Although *Dolania* populations do not appear to have a social structure, it is possible that other factors have created population bottlenecks capable of affecting heterozygosity levels. Such low population levels, however, would seem to be historical in nature (perhaps even founder events) because present population size for at least one of our study sites appears to be non-limiting. For example, based on Sweeney and Vannote's (1982) census data for emerging adult *Dolania* at site SC1, it appears that at least certain 100 meter reaches of river produce between 1000-1500 males and females capable of reproducing. Thus, unless *Dolania* populations within Upper Three Runs consist of an extremely large number of small isolated demes, it is unlikely that present heterozygosity levels are related to contemporary population size.

Although all populations of *Dolania* exhibited low heterozygosity levels, the SC1 population also exhibited fewer polymorphic loci (14.7%) than other populations (range: 23.5 - 31.0%). This difference does not appear to be a sampling problem because, for most loci, sample size for SC1 was significantly greater than for any of the other populations. However, sampling error cannot be ruled out completely, especially since missing alleles in the SC1 population were usually also rare (< 5% frequency for 5 of 6 loci) in occurrence elsewhere. The lower number of rare alleles in the SC1 populations, coupled with an extremely low level of heterozygosity, strongly suggests that this population has undergone an historical bottleneck which persisted for several generations and was followed by a gradual increase in population size (Maruyana and Fuerst, 1985 a, b).

The spatial and temporal data on *D. americana* at the two Blackwater River sites (FL1, FL2) are noteworthy from two aspects. First, little or no genetic variation was observed between two successive years at each site. Thus, there was no indication that the two year life cycle has resulted in two cohorts at a site which are genetically segregated. Second, spatial differences in allele frequencies at the *Hex* locus were significant (arc sine test) among the Blackwater River sites (FL1 vs. FL2). Although the differences were not large, they were very consistent for 1986 and 1987. These data suggest either some form of selection at this locus and/or a nonpanmictic breeding situation within the river system.

Final considerations

Although the biochemical evidence supporting the presence of a cryptic species at the Yellow River site in Alabama is convincing, there is presently no morphological basis for distinguishing *D. americana* from *D.* sp. nov. (Peters and Peters, personal communication). However, the Yellow River population of *D.* sp. nov. did exhibit significant differences in fecundity (average number of eggs = 169 ± 34, n = 9, AL1-87) relative to the nearby Blackwater River populations (107 ± 27, n = 15, FL1-87 and FL2-87; Peters and Peters, personal communication). Our data do not fully support the initial working hypothesis of this study. Although we observed significant genetic differentiation among certain *Dolania* populations, this variation appears to be largely interspecific. We did find statistically signifi-

cant genetic differentiation among *D. americana* populations in Florida and South Carolina. However, the level of this differentiation ($F_{st} = 0.059$) is within the range of values estimated from comparisons of distant populations of other stream mayflies (Sweeney et al., 1987).

An important ecological question remains to be resolved. Specifically, why does *D. americana* predominate in the Blackwater River while *D.* sp. nov. predominates in the nearby Yellow River? Although both are sandy bottomed, blackwater coastal plain streams, they differ significantly in size and discharge. For example, mean daily discharge for the Blackwater River between the FL1 and FL2 study sites was 8.4 cms (298 cfs) for calendar year 1986 and 26.2 cms (926 cfs) for the Yellow River at a point downstream of study site AL1 but above the confluence of the Shoal River (USGS, 1987). We hypothesize that the two *Dolania* species segregate ecologically within a given river system on factors that correspond to channel size and/or discharge; *D. americana* occupying intermediate size streams and rivers while *D.* sp. nov. occurs mainly in larger river channels. This hypothesis is based largely on our observations concerning the distribution of sibling species for other genera of aquatic insects within large river systems in eastern North America. Thus, although the watershed of a large river usually contains more than one species of various genera of mayflies (e.g. *Stenonema, Eurylophella, Ephemerella),* caddisflies (hydropsychids), or stoneflies *(Taeniopteryx),* a given species usually predominates in either small, intermediate, or large river channels but never in all three of these habitats (unpublished data). We predict that *Dolania* in the Yellow River will consist largely of *D. americana* in the smaller tributaries adjacent to or upstream of site AL1 but this species will be completely absent from the main river near its confluence with the estuary.

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