Porter, A. H. and E. M. Jakob. 1990. Allozyme variation in the introduced spider, *Holocnemus pluchei* (Araneae, Pholcidae) in California. J. Arachnol., 18:313-319.

ALLOZYME VARIATION IN THE INTRODUCED SPIDER *HOLOCNEMUS PLUCHEI* (ARANEAE, PHOLCIDAE) IN CALIFORNIA

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ABSTRACT

Ten electrophoretic loci were scored for five California populations of the pholcid spider, *Holocnemus pluchei*. Two loci were variable, with two alleles present at each. Genetic differentiation among populations was weak (mean $F_{ST} = 0.116$; Nei's unbiased $D \leq 0.015$); this may be attributable to the recency of introduction and opportunities for gene flow afforded by the affinity of these spiders for urban habitats. A single population of the ecologically similar pholcid *Pholcus phalangioides* differed from *Holocnemus* at seven of 10 loci.

INTRODUCTION

The Mediterranean pholcid spider *Holocnemus pluchei* (Scopoli) was recently introduced into the United States. The oldest reliable North American record known to us is an observation by W. R. Icenogle in Sutter Co., California in 1974 (S. Frommer pers. comm.). It is quite possible that *Holocnemus* was introduced into the state prior to 1974 but escaped attention because it superficially resembles another pholcid, *Pholcus phalangioides* (Fuesslin). In California, *Holocnemus* occurs in high densities below 500 m elevation in cities and towns in southern California and in the Central Valley. It is particularly common around buildings, and liable to be transported passively in truck and railroad cargo. We have seen small colonies as far east as Las Cruces, New Mexico.

Jakob and Dingle (1990) found statistically significant differences in development time and body size among broods of *H. pulchei* reared under identical conditions. Spiders in the field also show a wide range of phenotypic behavioral variation, including solitary living and group living (Jakob 1989, 1991). Here we report the genetic population structure of *Holocnemus* in California; the elucidation of genetic differentiation within and among

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Figure 1.—Collecting localities for *Holocnemus pluchei* in California.

populations provides an important context in which to study evolutionary processes. Because material was readily available, we also report the genetic distance between *Holocnemus* and *Pholcus phalangioides*, a phenotypically and ecologically similar spider also introduced from Europe.

METHODS

The *Holocnemus* populations surveyed are shown in Fig. 1; these were collected from university campuses and apartment buildings at five sites in California. *Pholcus* were collected in Wisconsin and mailed to Davis. In addition, *Holocnemus* broods reared from field collected egg sacs were assayed at polymorphic loci for evidence of Mendelian ratios, as an indication that the electromorphs represented heritable variants. All spiders were starved for one week prior to analysis to ensure that prey enzymes would be fully digested.

We used the electrophoresis protocol of Ayala et al. (1972). Thirteen enzyme systems were surveyed (Table 1). The computer program BIOSYS-1 (Swofford

Table 1.—Enzyme systems surveyed, with Enzyme Commission Numbers.

Enzyme	Abbreviation	E.C. #
Adenylate kinase	AK	2.7.4.7
Aldolase	ALDO	4.1.2.13
Fumarase	FUM	4.2.1.2
Glutamic-oxaloacetic transaminase	GOT	2.6.1.1
Glyceraldehyde-3-phosphate dehydrogenase	GAPDH	1.2.1.12
α-Glycerophosphate dehydrogenase	α-GPD	1.1.1.8
Hexokinase	HK	2.7.1.1
Isocitrate dehydrogenase	IDH	1.1.1.42
Malate dehydrogenase	MDH	1.1.1.37
Malic enzyme	ME	1.1.1.40
Phosphoglucose isomerase	PGI	5.3.1.9
Phosphoglucomutase	PGM	2.7.5.1
Superoxide dismutase	SOD	1.15.1.1

and Selander 1981) was used for the genetic analyses. χ^2 procedures were used to test for deviations from Hardy-Weinberg expectations. Genetic variability scores (heterozygosity and polymorphic loci) provide an estimate of the degree of variation available for evolutionary change in populations. We report two standard heterozygosity scores: observed heterozygosity (H_{obs}) is the proportion of loci found to be heterozygous by direct observation of genotypic frequencies; expected heterozygosity (H_{exp}) is the proportion of heterozygotes calculated from allelic frequencies under the expectation of Hardy-Weinberg ratios of genotypic frequencies. We also report the percent of loci we observed to be polymorphic (P) in each population, and provide a rough comparison of these statistics to those of other spiders.

Divergence among populations was analyzed using Nei's (1978) unbiased genetic distance, which adjusts for small and variable sample sizes, and also using Wright's (1931) F_{ST}. F_{ST} is an estimate of the component of overall genetic variance attributable to among-population effects, standardized by the total genetic variance available. F_{ST} can be related directly to important homogenizing and differentiating influences of gene flow, natural selection, and genetic drift. Differentiation is strong when $F_{ST} > 0.33$: above this level, the effects of homogenizing factors (gene flow and balancing selection) become relatively unimportant in determining differences among populations (see Wright [1978] and Slatkin [1985] for discussion). The mathematical definitions of the population genetic parameters reported here can be found in any introductory population genetics textbook (e.g., Hedrick 1985).

RESULTS AND DISCUSSION

We were able to stain and reliably score 10 loci (GAPDH, GOT-1, GOT-2, HK, IDH-1, MDH-1, MDH-2, PGI, PGM, and 6-PGD; where "1" is the fastest locus migrating in the cathodal direction). In *Holocnemus*, two of these loci were variable (GOT-1, PGI) with two alleles each; the remainder were fixed for the same allele in all populations. Allelic frequencies for the variable loci are given in Table 2; genotypic frequencies did not deviate from Hardy-Weinberg expectations. The reared broods assayed for GOT-1 and PGI showed Mendelian

Table 2.—Animals sampled (*N*) and allelic frequencies for variable loci in *Holocnemus pluchei* populations. Allele F migrates fast cathodally, S is slower.

		Locus and allele			
		GOT-1		PGI	
Population	*N*	F	S	F	S
Davis	29	0.603	0.397	0.879	0.121
Fresno	20	0.684	0.316	0.975	0.025
Bakersfield	19	0.947	0.053	0.765	0.235
Newhall	15	1.000	0.000	0.893	0.107
Riverside	19	0.816	0.184	0.971	0.029

ratios in most cases where variability was present (Table 3). However, Brood 1 deviated from Mendelian ratios at GOT-1; this may have been due to multiple mating with males of different GOT-1 genotypes, but if so, the genotypic ratio at PGI indicates that all the fathers were PGI heterozygotes. No field data concerning the frequency of multiple mating are available.

Genetic variability scores for all populations are shown in Table 4. Genetic distances between *Holocnemus* populations are quite low (Table 5), and analysis using F_{ST} indicates that the relative genetic differentiation among populations is biologically minor (GOT-1: $F_{ST} = 0.148$; PGI:$F_{ST} = 0.063$; mean $F_{ST} = 0.116$). As a comparison, mean $F_{ST} = 0.009$ among sample populations of the eastern North American monarch butterfly (Eanes and Koehn 1978), which is essentially panmictic; $F_{ST} = 0.705$ among sample populations of a plethodontid salamander (Wake and Yanev 1986). The genetic distance between *Pholcus* and *Holocnemus* is high (Table 5): these taxa show fixed differences at seven of the ten loci scored (GAPDH, GOT-2, HK, IDH-1, MDH-1, PGI, 6-PGD), suggesting a very old divergence between these ecologically rather similar species.

While genetic variability in *Holocnemus pluchei* is low relative to most invertebrate species examined (Nevo 1978), it remains within the range reported in other spiders. Different heterozygosity parameters used in the arachnological literature makes comparison difficult, permitting only a rough sense of the reported range of variability: heterozygosities (H_{obs} and H_{exp}) from the literature range from a low of 0.017 in *Anelosimus eximius* (H_{exp}; Smith 1986) to a high of 0.094 in *Araneus ventricosus* (H_{obs}; Manchenko 1981). The degree of

Table 3.—Genotypic frequencies for variable loci in broods reared from wild-collected females. Only brood 6 at GOT-1 differs significantly from Mendelian expectations ($P < 0.0001$; see text).

	1989 brood number				
Genotype	6	7	8	9	10
GOT-1					
FF	23	8	-	6	10
FS	15	-	10	4	-
SS	-	-	-	-	-
PGI					
FF	11	8	10	10	5
FS	17	-	-	-	5
SS	10	-	-	-	-

Table 4.—Genetic variability scores for all populations. A = mean number of alleles per locus; H_{obs} = observed proportion of heterozygotes; H_{exp} = proportion of heterozygotes calculated from Hardy-Weinberg proportions; P = percent of loci polymorphic, with more than one allele detected. Standard errors in parentheses.

Population	A	H_{obs}	H_{exp}	P
Davis	1.2 (0.1)	0.083 (0.061)	0.070 (0.051)	20.0
Fresno	1.2 (0.1)	0.068 (0.063)	0.049 (0.044)	20.0
Bakersfield	1.2 (0.1)	0.046 (0.036)	0.047 (0.037)	20.0
Newhall	1.1 (0.1)	0.021 (0.021)	0.020 (0.020)	10.0
Riverside	1.2 (0.1)	0.043 (0.037)	0.037 (0.031)	20.0
Wisconsin (*Pholcus*)	1.0 (0.0)	0.000 (0.000)	0.000 (0.000)	0

polymorphism (assessed as the percent of loci with more than one electromorph observed) ranges from a low of 3.9% in one population of *A. eximius* (Smith 1986) to a high of 33% in an *A. ventricosus* population (Manchenko 1981). We omit the high variability scores calculated from Pennington's (1979) genotypic frequency data because he assayed only polymorphic loci. Note however that it is not possible to generalize about variability across all spiders because most previous work concerns spiders with unusual social structures that may well influence patterns of genetic variability (see also Cesaroni et al. 1981). The high genetic similarity among *Holocnemus* populations may have up to three contributing factors. If natural selection on these loci is negligible, genetic drift alone countered by a gene exchange rate of approximately 2 individuals per generation will explain the observed level of population differentiation (using Wright's [1931] formulation $Nm \approx (1/F_{ST} - 1)/4$, where Nm is the rate of gene exchange among populations in an island model of genetic population structure; see also Slatkin and Barton [1989]). This level of gene flow is well within the range expected from the spiders' affinity for urban and suburban habitats. However, selection for balanced polymorphisms at variable loci can also promote similarity. The recency of the *Holocnemus* introduction in California may promote similarity as well: genetic drift is a function of population size, and the large population sizes in California may not have had time to fully differentiate. These latter factors, depending on their importance, will correspondingly reduce the estimate of gene flow required to explain present levels of differentation. Repetition of this study after 10-15 years, and a study of European populations, would help to determine the relative importance of these factors.

Holocnemus is also unusual in having been recently introduced in California, and its low variability scores are perhaps to be expected: low heterozygosity in founder populations is well known (e.g., Harrison et al. 1983). Indeed, the

Table 5.—Pairwise genetic distances between populations using Nei's (1978) unbiased genetic distance.

Population	Davis	F	B	N	R
Fresno (F)	0.000				
Bakersfield (B)	0.013	0.011			
Newhall (N)	0.015	0.010	0.001		
Riverside (R)	0.004	0.001	0.005	0.003	
Pholcus	1.309	1.290	1.197	1.194	1.249

maximum of two alleles per locus found in this survey suggests that the original California propagule may have been as small as a single gravid female. The complete lack of genetic variability in the single *Pholcus* population may not be representative of the species as a whole, because this sample was collected from a small, isolated population.

Given the relatively low genetic variability scores and the recency of introduction into California, the differences in life history traits among families reared under identical conditions (Jakob 1989; Jakob and Dingle 1990) are striking. Such variation may result from genetic differences among families, but may also arise in part from differences in the maternal environment during egg maturation—egg size, for example, may vary depending on the mother's foraging success. Maternal effects can be quantified through more elaborate experimental designs. The wide range of behavior expressed during *H. pluchei* social interactions in the field (Jakob 1989, 1991) may be maintained in the population by genetic polymorphisms in loci which regulate such behaviors deterministically, or a "general purpose" genotype shared by all members of the population which permits the spiders to behave flexibly. To the extent that the low level of genetic variability shown in this study is representative of the genome, the second alternative seems most likely.

The low variability in the loci studied does not bode well for the use of electrophoretic data for *in situ* paternity analysis or other fine-grained field studies in *Holocnemus* (c.f., Jakob 1989). However, this technique could be used under laboratory conditions to determine, for example, whether spiderlings joining groups prefer closely related individuals.

ACKNOWLEDGMENTS

We thank H. Dingle, F. J. Ayala, and H. B. Shaffer for the use of laboratory facilities; H. Dingle, P. S. Ward, J. Stamps, M. G. Ramirez, and Y. D. Lubin for comments on the manuscript; S. Frommer, D. Ubick and M. Moody for *Holocnemus* identification and unpublished information on its introduction; T. Reichert for the *Pholcus* sample; and A. Lung, D. Cook, and N. Gregory for help with rearing. Jastro-Shields Scholarships to both authors from the University of California at Davis and a Sigma Xi Grant-in-Aid of Research to EMJ covered electrophoresis and travel costs.

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Manuscript received January 1990, revised May 1990.