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## GENETIC DIFFERENTIATION AMONG THREE SPECIES OF *PARDOSA* (ARACHNIDA: LYCOSIDAE)

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### ABSTRACT

Allozymic variation in nine protein producing loci was examined in three species of *Pardosa* using starch gel electrophoresis. Allozyme frequencies showed a high degree of geographic uniformity among conspecific populations. Estimated heterozygosities for the three species ranged from 0.05 to 0.15. Rogers' coefficients of genetic similarity based on allozyme frequencies averaged over conspecific populations ranged from 0.16 to 0.37 for the three species.

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*Pardosa* are active wandering spiders of the family Lycosidae, the wolf spiders. About 50 species are known from North America, but many are morphologically similar and difficult to identify. This is especially true for the majority of species that occur in the western United States. Additional taxonomic characters would be valuable in reducing systematic confusion.

Allozymic variation in proteins is a useful tool for investigating systematic relationships of closely related species (Avisé 1974). Allozymic variation in the proteins commonly examined has a simple genetic basis. While it is recognized that more genetic variation may be present at most loci than can be detected by electrophoretic techniques, electrophoretic studies yield useful information concerning levels of genetic variation within and among species of animals and plants. In general, studies indicate a high degree of genetic similarity among conspecific populations. Nei (1978) has shown that it is often possible to adequately characterize the total allozymic variation within a species from one or a few population samples. Congeneric species generally show much lower levels of genetic similarity than do conspecific populations. Comparisons among congeneric species yield phenetic groupings which usually correspond closely with those derived by classical methods (Avisé 1974).

The purpose of this study is to assess the potential value of electrophoretic data as a taxonomic tool within the *Pardosa* and to compare the level of allozymic variability in the genus with levels of allozymic variability observed in other animal groups.

### MATERIALS AND METHODS

This study was based on 159 specimens representing three species of *Pardosa* collected during the summer of 1976. *Pardosa moesta* Banks was collected from four locations: Mott Preserve, Van Buren Co., MI, the Kalamazoo Nature Center, Kalamazoo Co., MI, roadside grass, Chippewa Co., MI, and City of Marquette, Marquette Co., MI. *Pardosa milvina* Hentz was sampled from two locations: Standing Stone State Park, Clay Co., TN, and Wildwood Trailer Park, Jackson Co., IL. *Pardosa distincta* Blackwall was collected from two locations: roadside grass, Luce Co., MI, and the City of Marquette, Marquette Co., MI. Specimens were collected within an area of approximately 100 m<sup>2</sup> and returned to the laboratory alive; they were then stored at -70°C until just prior to electrophoresis at which time the animals were individually homogenized in 0.1 ml 0.2 M Tris HCl, pH 7.8. The

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homogenate was absorbed onto two small rectangular pieces of No. 3 filter paper and applied to two horizontal starch gels. Electrophoresis and histochemical staining were performed as described by Selander et al. (1971) and Brewer (1970). Samples were assayed using two buffer systems. Isocitrate dehydrogenase (IDH-1 and IDH-2), lactate dehydrogenase (LDH),  $\alpha$ -glycerophosphate dehydrogenase ( $\alpha$ -GPD), and hexokinase (HK) were resolved using buffer system No. 5 (Selander et al., 1971). Phosphoglucose isomerase (PGI), phosphoglucomutase (PGM), and general protein (PT-2 and PT-4) were resolved using buffer system No. 2 (Selander et al. 1971). All proteins migrated anodally on the buffer systems used.

Allozymes at a particular locus were alphabetized according to their relative mobilities on the specified electrophoretic buffer systems, without regard for species; the fastest migrating allozyme was designated (a) while correspondingly slower allozymes were designated b, c, and so on. Determinations of identity and non-identity were made on the basis of side by side comparisons or near juxtaposition on the same gel. Standards containing the most common allozymes occurring at each protein system for *moesta* and *milvina* were placed at various locations on each gel to insure that frequently encountered allozymes were not misidentified. Low frequency allozymes at each protein for which direct comparisons could not be made were grouped into one class designated (other) in Table 1; allozymes in this class were not included in comparisons of genetic similarity among species.

Rogers' coefficient of genetic similarity (Rogers 1972) was used to estimate the degree of genetic similarity among the three species.

The genetic interpretation of our results was inferential since progeny studies were not undertaken. However, our results were supported by the general agreement of genotypic frequencies with those expected under conditions of Hardy-Weinberg equilibrium and by patterns of allozymic variation in other animal species.

Table 1. Sample size and allozyme frequencies for nine variable proteins in eight populations representing three species of *Pardosa*.

Protein Allozyme	Population <sup>a</sup>							
	1	2	3	4	5	6	7	8
Sample size	30	26	11	15	30	27	11	9
PGI								
Pgi <sup>a</sup>	.00	.00	.00	.00	.00	.00	.09	.17
Pgi <sup>b</sup>	.00	.00	.00	.00	.00	.00	.45	.05
Pgi <sup>c</sup>	.00	.00	.00	.00	.00	.13	.00	.00
Pgi <sup>d</sup>	.07	.08	.09	.07	.05	.00	.00	.00
Pgi <sup>e</sup>	.00	.00	.00	.00	.00	.00	.41	.78
Pgi <sup>f</sup>	.93	.90	.86	.90	.93	.83	.00	.00
other	.00	.02	.05	.03	.02	.04	.05	.00
PGM								
Pgm <sup>a</sup>	.00	.00	.00	.00	.00	.00	.09	.10
Pgm <sup>b</sup>	.95	.94	.94	.94	.00	.00	.32	.33
Pgm <sup>c</sup>	.00	.00	.00	.00	.00	.00	.55	.53
Pgm <sup>d</sup>	.00	.00	.00	.00	.93	.93	.00	.00
other	.05	.06	.06	.06	.07	.07	.04	.04
IDH-1								
Idh-1 <sup>a</sup>	1.00	1.00	.95	1.00	.03	.09	.90	.56
Idh-1 <sup>b</sup>	.00	.00	.00	.00	.90	.91	.00	.00
Idh-1 <sup>d</sup>	.00	.00	.00	.00	.00	.00	.05	.44
other	.00	.00	.05	.00	.07	.00	.05	.00

Table 1. (Continued)

Protein Allozyme	Population <sup>a</sup>							
	1	2	3	4	5	6	7	8
<b>IDH-2</b>								
Idh-2 <sup>a</sup>	.97	.92	.95	.90	.00	.00	.00	.00
Idh-2 <sup>b</sup>	.00	.00	.00	.00	.00	.00	1.00	1.00
Idh-2 <sup>c</sup>	.03	.04	.05	.10	1.00	1.00	.00	.00
Idh-2 <sup>d</sup>	.00	.04	.00	.00	.00	.00	.00	.00
<b>α-GPD</b>								
α-Gpd <sup>a</sup>	1.00	1.00	1.00	1.00	.00	.00	.00	.00
α-Gpd <sup>b</sup>	.00	.00	.00	.00	.97	.98	.00	.00
α-Gpd <sup>c</sup>	.00	.00	.00	.00	.00	.00	1.00	1.00
other	.00	.00	.00	.00	.03	.02	.00	.00
<b>LDH</b>								
Ldh <sup>a</sup>	.97	1.00	1.00	.93	.00	.26	.00	.00
Ldh <sup>b</sup>	.00	.00	.00	.00	.13	.00	.00	.00
Ldh <sup>d</sup>	.00	.00	.00	.00	.85	.74	1.00	1.00
other	.03	.00	.00	.07	.02	.00	.00	.00
<b>HK</b>								
Hk <sup>a</sup>	1.00	.98	1.00	1.00	.00	.00	.00	.00
Hk <sup>b</sup>	.00	.00	.00	.00	.97	1.00	1.00	1.00
other	.00	.02	.00	.00	.03	.00	.00	.00
<b>PT-2</b>								
Pt-2 <sup>a</sup>	1.00	1.00	1.00	1.00	1.00	1.00	.00	.00
Pt-2 <sup>b</sup>	.00	.00	.00	.00	.00	.00	1.00	1.00
<b>PT-4</b>								
Pt-4 <sup>a</sup>	1.00	1.00	1.00	1.00	1.00	1.00	.00	.00
Pt-4 <sup>b</sup>	.00	.00	.00	.00	.00	.00	1.00	1.00

<sup>a</sup>1—*P. moesta*, Mott Reserve, Van Buren Co., MI; 2—*P. moesta*, Kalamazoo Nature Center, Kalamazoo Co., MI; 3—*P. moesta*, roadside grass, Chippewa Co. MI; 4—*P. moesta*, City of Marquette, Marquette Co., MI; 5—*P. milvina*, Standing Stone State Park, Clay Co., TN; 6—*P. milvina*, Wildwood Trailer Park, Jackson Co., IL; 7—*P. distincta*, roadside grass, Luce Co., MI; 8—*P. distincta*, City of Marquette, Marquette Co., MI.

## RESULTS AND DISCUSSION

Allozyme frequencies and sample size for the nine proteins studied are shown in Table 1. One allozyme predominated in all populations of a species at most proteins; however, in *distincta* two allozymes were present in varying frequencies at PGI and IDH-1. There was some sharing of allozymes among species, i.e. Idh-1<sup>a</sup> was found in all three species and Pgi<sup>d</sup>, Pgi<sup>f</sup>, Pt-2<sup>a</sup>, Pt-4<sup>a</sup>, Idh-2<sup>c</sup>, Hk<sup>b</sup>, Ldh<sup>a</sup>, Ldh<sup>c</sup>, and Pgm<sup>b</sup> were found in two species.

The geographic pattern of allozyme frequencies within each of the three species showed a high degree of uniformity. This pattern is typical for animal species with high dispersal abilities (Soule 1976). *Pardosa* are active dispersers with frequent observation of ballooning in which the spiders spin out silk and are picked up and carried long distances by the wind.

The estimated average levels of heterozygosity (H) based on the nine proteins studied were 0.05, 0.10, and 0.15 for *moesta*, *milvina*, and *distincta*, respectively. Because levels of heterozygosity vary widely among loci, estimates of H based on small numbers of loci must be interpreted cautiously. However, it has become apparent from the numerous studies that have been completed that not all types of organisms are equally variable (Selander 1976).

Table 2. Rogers' coefficients of genetic similarity among three species of *Pardosa*.

	<i>moesta</i>	<i>milvina</i>	<i>distincta</i>
<i>moesta</i>	1.000	0.371	0.157
<i>milvina</i>	0.371	1.000	0.260
<i>distincta</i>	0.157	0.260	1.000

The estimated level of heterozygosity for the three species of *Pardosa* ( $\bar{H} = 0.10$ ) was similar to that found for other invertebrate species groups (Nevo 1978).

Estimates of genetic similarity ( $S$ ) among the three species ranged from 0.16 to 0.37 (Table 2) with a mean across species ( $\bar{S}$ ) of 0.27. Most groups of closely related congeneric species studied show somewhat higher estimates of  $\bar{S}$  (Avice 1974). The low estimate of  $\bar{S}$  for the three *Pardosa* species may have been due to the chance selection of proteins for our study which were highly differentiated in *Pardosa*. Perhaps the study of additional proteins would have resulted in an increased estimate of average genetic similarity. Alternatively, the low estimate of  $\bar{S}$  for the three species may reflect a truly high level of genetic divergence among the species in spite of considerable external morphological similarity. All three species occur in similar habitats and it is possible that selection for similar external morphology has obscured a high level of genetic divergence among the species.

In our study  $\alpha$ -GPD served as a diagnostic taxonomic criterion for differentiating the three species, since no two species shared common allozymes at that locus, while the probability of misclassifying a species using IDH-2 as a criterion was no greater than 0.01. If a high level of divergence in allozymes is characteristic of the genus as a whole, it is conceivable that relatively few protein-producing loci could serve as a taxonomic basis for differentiating species in the genus.

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