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Genetic Structure in Korean Populations of *Atractomorpha lata* (Orthoptera: Pyrgomorphidae)

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Allozyme variation of seven enzyme systems was analyzed from 202 individuals from four Korean populations of *Atractomorpha lata*. These populations exhibit higher levels of allozyme variation than average values of in most other insects with a mean 64% of polymorphic loci and a mean 0.384 of expected heterozygosity within populations. Fixation indices indicated considerable substructuring within populations sampled (mean F_{IS}=0.403), indicating probable inbreeding or assortative mating coupled with restricted migration between subpopulations. This was supported by the field observation that the species exists as small, discrete colonies in meadow habitats and females carry males. In addition, significant differences in allele frequencies between males and females at polymorphic loci examined (70%, 16 of 23 cases) could account for the observed heterozygote deficiencies.

Studies of Orthopteran species (Moran et al., 1980; Gill, 1981; Hamrick and Hamrick, 1989) revealed that their genetic variability within populations was substantially higher than in most populations of vertebrates and plants (Nevo, 1978; Nevo et al., 1984; Hamrick and Godt, 1989). However, there are only a few studies on population genetic structure of Orthopteran species. Thus, it might be necessary to investigate genetic variation and population genetic structure of this group. In addition, very little is known of genetic variation of insects in East Asia, particularly in Korea (Chung et al., 1997).

Atractomorpha lata Motschlsky (Orthoptera: Pyrgomorphidae) is distributed from central and southern Korean Peninsula and Japan into Taiwan and China (Ito et al., 1980) commonly found in meadows and along field margins. Mature females (42 mm long) are larger than males (25 mm long) and both sexes are green or grayish brown. There is typically one generation per year in Korea. As the flight distance of the species is short (ca. a few meters, Chung, pers. obs.), and if a female mates selectively with a particular male, it is predicted that an overall deficiency of heterozygosity exists within a population (population substructuring). Population structure could be affected by partial inbreeding, the Wahlund effect caused by restricted neighborhood size from limited gene flow (Hartl and Clark, 1989), the difference in gene frequency between males and females (Hamrick and Hamrick, 1989), microenvironmental selective pressure, if any, and the historical events (Brown, 1979). In an attempt to understand better the biology of *Atractomorpha lata*, we have started to estimate 1) levels and distribution of genetic variation within and among populations of the species, 2) Wright's (1922) fixation indices, and 3) the differences in allele frequency between males and females.

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Materials and Methods

A total of 202 adult individuals from four populations or localities in Korea was collected. Population codes and sample sizes are presented in Fig. 1. Living specimens were brought to the laboratory and stored in a refrigerator. To extract enzymes the gut was removed and the remaining tissues were homogenized adding a phosphate buffer described by Mitton et al. (1979). Electrophoresis was performed using 10.5% starch gels. Phosphoglucomutase (PGM), phosphoglucose isomerase (PGI), malate dehydrogenase (MDH), shikimate dehydrogenase (SKDH), and peroxidase (PER) were resolved on a modification (Chung and Kang, 1994) of Soltis et al. (1983) system 11. Poulik buffer system, a modification (Haufler, 1985) of Soltis et al. (1983) system 8 was used to resolve triosephosphate isomerase (TPI) and fluorescent esterase (FE). All stain recipes were identical to those described by Soltis et al. (1983).

For enzymes with more than one zone of activity, the most anodally migrating zone was designated as locus '*1*', the next as locus '*2*', etc. Likewise, alleles were designated sequentially, with the most anodally

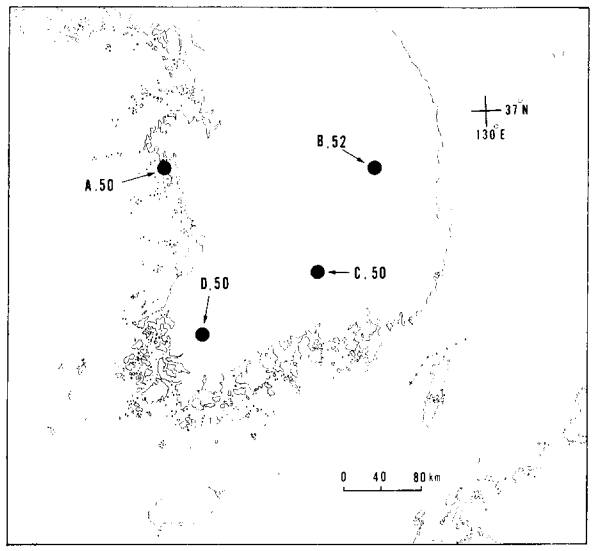

Fig. 1. The locations of the four sampled populations of *A. lata* in Korea. Sample sizes and population codes are indicated for each population. A, Prov. Chungcheongnam, Taean gun, Anmyeon eup, near Bangpo Beach (30×100-m area); B, Prov. Gyeongsangbuk, Andong shi, Gilan myeon, Cheonji ri (20×60-m area); C, Prov. Gyeongsangnam, Sanchung gun, Shinan myeon, Dangae ri (30×70-m area); and D, Prov. Chollanam, Naju shi, Nampyeong eup, Pungrimri (20×65-m area).

migrating allele designated '*a*'. A locus was considered polymorphic if two or more alleles were observed, regardless of their frequencies. The polymorphic loci seemed to be consistent with Mendelian inheritance. Electrophoretic banding patterns for six enzymes are presented in Fig. 2. FE was not included in Fig. 2 because *Fe-1* was monomorphic screened for all individuals and *Fe-2* and *Fe-3* were poorly resolved. Four genetic parameters were estimated using a computer program developed by M. D. Loveless and A. Schnabel (pers. comm.): percent polymorphic loci (P), mean number of alleles per locus (A), effective number of alleles per locus (Ae), and expected heterozygosity or gene diversity (He). The statistics of the these parameters were described by Hartl and Clark (1989). Observed heterozygosity was compared to Hardy- Weinberg (H-W) expected values using Wright's (1922) fixation indices (F) or the inbreeding coefficients. We tested for significant deviations at each polymorphic locus and in each population using Chi-square tests following Li and Horvitz (1953). In addition, Wright's (1965) F-statistics (F_{IT}, F_{IS}, and F_{ST}) were also used to analyze genetic structure in *Atractomorpha lata*. Deviations of F_{IT} and F_{IS} from zero were also tested using the Chi-square statistic (Li and Horvitz, 1953). Statistical significance of each F_{ST} value was calculated by Chi-square test (Workman and Niswander, 1970). Allele frequencies were calculated separately for males and females and tested with a heterogeneity Chi-square test for significant differences. In addition, Wright's (1922) fixation indices were analyzed separately for males and females to determine whether heterozygote deficiency is due to mixing sexes.

Table 1. Allele frequencies at six polymorphic loci estimated in four populations of *Atractomorpha lata* from Korea. Population codes and sample sizes as in Fig. 1

Locus	Allele	A	B	C	D
Pgm	*a*	0.000	0.000	0.040	0.030
	b	0.360	0.346	0.270	0.220
	c	0.090	0.000	0.000	0.000
	d	0.300	0.183	0.390	0.540
	e	0.070	0.202	0.080	0.090
	f	0.180	0.269	0.190	0.080
	g	0.000	0.000	0.030	0.040
Pgi	*a*	0.170	0.212	0.000	0.040
	b	0.270	0.596	0.000	0.080
	c	0.560	0.192	1.000	0.880
Mdh-1	*a*	0.390	0.385	0.220	0.260
	b	0.090	0.000	0.000	0.080
	c	0.520	0.615	0.780	0.660
Mdh-2	*a*	0.000	0.000	0.180	0.000
	b	0.240	0.385	0.180	0.370
	c	0.020	0.000	0.040	0.100
	d	0.720	0.615	0.380	0.310
	e	0.020	0.000	0.220	0.220
Skdh-1	*a*	0.060	0.019	0.049	0.060
	b	0.280	0.327	0.314	0.230
	c	0.180	0.077	0.343	0.290
	d	0.180	0.115	0.059	0.140
	e	0.300	0.462	0.235	0.280
Per-2	*a*	0.370	0.211	0.200	0.120
	b	0.500	0.654	0.488	0.200
	c	0.000	0.058	0.170	0.240
	d	0.130	0.077	0.150	0.440

Results

Seven enzymes, encoded by 12 putative loci, were detected. *Skdh-2, Fe-2* and *Fe-3* were expressed but not scored because of poor activity and/or resolution. *Per-1, Tpi*, and *Fe-1* were monomorphic in all populations. Allele frequencies at the six polymorphic loci (*Pgm, Pgi, Mdh-1, Mdh-2, Skdh-1,* and *Per-2*) are presented in Table 1. The mean number and the mean effective numbers of alleles per locus were 2.84 and 2.15, respectively (Table 2), suggesting that many alleles were present at a more or less even frequency. The mean expected heterozygosity (He) was 0.384, while estimates of observed individual

Table 2. Genetic variability at nine loci in four populations of *A. lata**

Pop**	P	Ae	A	Ho (SE)	He (SE)
A	66.7	2.22	2.78	0.218 (0.017)	0.408 (0.036)
B	66.7	1.98	2.56	0.239 (0.017)	0.380 (0.033)
C	55.6	2.20	2.89	0.255 (0.017)	0.358 (0.028)
D	66.7	2.22	3.11	0.251 (0.018)	0.391 (0.040)
Mean	63.9	2.15	2.84	0.241 (0.013)	0.384 (0.028)

* Abbreviations P: percentage of polymorphic loci; A: mean number of alleles per locus; Ae: effective number of alleles per locus; Ho: observed heterozygosity; and He: Hardy-Weinberg expected heterozygosity or genetic diversity.
** Population code as in Fig. 1.

Table 3. Wright's fixation indices for four populations of *A. lata*. The dash (Population C on Pgi) indicates a monomorphic locus. The indices were calculated by mixing sexes (T) and separating males (M) and females (F). Sample size (M/F) per population is indicated in parentheses. Test significant at the 95% (*), or 99.9% (***) level; ns=not significant

Locus	A (25/25)	B (28/24)	C (25/25)	D (26/24)
Pgm				
T	0.434***	0.273ns	0.349*	0.119ns
M	0.272ns	0.327ns	0.004ns	0.278ns
F	0.625***	0.212ns	0.590***	0.118ns
Pgi				
T	0.831***	0.662***	-	0.636***
M	0.906***	0.843***	-	0.446*
F	0.760***	0.478*	-	0.595***
Mdh-1				
T	0.687***	0.517***	-0.039ns	0.117ns
M	0.720***	0.524***	-0.075ns	0.160ns
F	0.569**	0.818***	0.020ns	0.263ns
Mdh-2				
T	0.439***	0.356**	0.412***	0.525***
M	0.588***	-0.031ns	0.170ns	0.580***
F	0.315ns	0.674***	0.596***	0.350ns
Skdh				
T	0.300ns	0.250ns	0.328*	0.402**
M	0.402ns	0.382ns	0.441*	0.553***
F	0.101ns	0.060ns	0.256ns	0.140ns
Per-2				
T	0.203ns	0.265ns	0.241ns	0.401***
M	0.113ns	0.108ns	0.074ns	0.486**
F	0.262ns	0.366ns	0.390ns	0.082ns

heterozygosity were less than expected (mean Ho= 0.241), indicating an overall deficiency of heterozygotes within the populations.

Analysis of fixation indices, calculated for all polymorphic loci in each population, showed substantial deficiencies of heterozygotes relative to H-W expectations (Tables 3 & 4). Except for *Mdh-1* and population C, all fixation indices were positive, and 14 (61%) of these departed significantly from zero ($P<0.05$), indicating a deficiency of heterozygosity at those loci and in those populations (Table 3). On the other hand, when males and females were analyzed separately, a smaller number of the significant positive fixation indices were observed (41%, 10/23 for males and 39%, 9/23 for females, Table 3). Wright's *F*-coefficients showed that significant deficiencies of heterozygotes exist for all polymorphic loci at the level of population (mean F_{IS}=0.403) and the samples as a whole (mean F_{IT}=0.448) (Table 4). About 70% of all cases tested (16/23) departed significantly from zero

Table 4. The estimates of Wright's F_{ST}, F_{IS}, and F_{IT} for six polymorphic loci in *A. lata*. All estimates of F_{ST}, F_{IS}, and F_{IT} were significant at 99.9% level

Locus	# alleles	F_{ST}	F_{IS}	F_{IT}
Pgm	7	0.041	0.310	0.338
Pgi	3	0.319	0.726	0.813
Mdh-1	3	0.033	0.356	0.378
Mdh-2	5	0.084	0.433	0.481
Skdh-1	5	0.029	0.315	0.335
Per-2	4	0.097	0.274	0.345
Mean	4.5	0.100	0.403	0.448

Table 5. Statistical tests of allele frequency differences between males and females. The dash (Population C on *Pgi*) indicates a monomorphic locus. Test significant at the 95% (*), 99% (**), or 99.9% (***) level

Locus	A	B	C	D
Pgm	13.6*	22.2**	19.9**	n.s.
Pgi	11.8**	25.6***	-	n.s.
Mdh-1	13.8**	10.7**	n.s.	n.s.
Mdh-2	n.s.	n.s.	25.8***	23.7***
Skdh-1	27.9***	12.2*	9.7*	13.7**
Per-2	n.s.	18.1**	11.5**	19.5***

($P<0.05$), indicating significant differences in allele frequencies between males and females (Table 5). Although overall 90% of the total variance in the species is common to all populations (mean F_{ST}= 0.100), significant differences in allele frequencies among populations were found for all polymorphic loci ($P<0.001$) (Table 4).

Discussion

Korean populations of *A. lata* have levels of genetic variability comparable to those of another orthopteran species (Hamrick and Hamrick, 1989) and two odonatan species (Chung et al., 1997), and higher than those of most populations of vertebrates and plants (Nevo, 1978; Nevo et al., 1984; Hamrick and Godt, 1989). However, a significant deficiency of heterozygotes within populations was revealed by all positive values of F_{IS} for all loci. This can result from inbreeding (Wright, 1978), or from pooling of subpopulations differing in allele frequency (Wahlund effect, Wahlund, 1928). As movement of *A. lata* individuals is limited (Chung, pers. obs.) and a male is carried on the back of a female (Ito et al., 1980), it is highly probable that some form of non-random mating might occur within populations sampled. To conform this assumption, a mark and recapture study should be necessary to quantify the neighborhood size and movement within and among habitats. Although individuals were found to be aggregated and patchy in distribution on the meadows and along field margins, samples were collected from several patches per population. The samples within a population or locality were pooled in this study. If these aggregates represent subpopulations differing to some extent in allele frequency, pooling them for electrophoretic study would result in a Wahlund effect.

Significant differences in allele frequencies between males and females (ca. 70%) could also account for the observed heterozygote deficiencies for those loci (genetic substructuring within a population). In addition, a smaller number of the significant positive fixation indices for males (10) and females (9) was observed than for mixing sexes (14). These indicated that the difference in allele frequencies between the sexes was one of the major factors responsible for the observed heterozygote deficiency for most polymorphic loci in each population. At present, it is not well

understood why a significant difference in allele frequencies between males and females is present in this species. Biology of this species such as females carrying males, dispersal behavior, and time of reproduction, etc., may be possible factors. None of the polymorphic loci can be sex-linked because heterozygotes occurred in both sexes.

The overall results showed that 10% of the total variance of allele frequencies was due to genetic differentiation among populations. The difference in allele frequencies among populations were in fact significant for all loci. This is what would be expected for this species living usually in isolated meadow habitats in Korea between which there is limited migration.

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